PCT

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :		(11) International. Publication Number:	WO 92/01059
C12P 21/08, A61K 39/395 C12N 15/13, C07K 15/28 .	A1	(43) Internati nal Publication Date:	23 January 1992 (23.01.92)

(21) International Application Number: PCT/GB91/01108

(22) International Filing Date: 5 July 1991 (05.07.91)

(30) Priority data:
9014932.9   5 July 1990 (05.07.90)   GB
PCT/GB90/02017   21 December 1990 (21.12.90) WO
(34) Countries for which the regional
    or international application
    was filed: GB et al.

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(81) Designated States: AT, AT (European patent), AU, BB, BE (European patent), BF (OAPI patent), BG, BJ (OAPI patent), BR, CA, CF (OAPI patent), CG (OAPI patent), CH, CH (European patent), CI (OAPI patent), CM (OAPI patent), CS, DE, DE (European patent), DK, DK (European patent), ES, ES (European patent), FI, FR (European patent), GA (OAPI patent), GB, GB (European patent), GN (OAPI patent), GR (European patent), HU, IT (European patent), JP, KP, KR, LK, LU, LU (European patent), MC, MG, ML (OAPI patent), MN, MR (OAPI patent), MW, NL, NL (European patent), NO, PL, RO, SD, SE, SE (European patent), SN + (OAPI patent), SU, TD (OAPI patent), TG (OAPI patent), US.

Published
With international search report.

(54) Title: CDR GRAFTED ANTI-CEA ANTIBODIES AND THEIR PRODUCTION

(57) Abstract

The present invention provides humanised antibody molecules (HAMs) having specificity for carcinoembryonic antigen (CEA) and having an antigen binding site wherein at least one of the complementarity determining regions (CDRs) of the variable domains is derived from the mouse monoclonal antibody A5B7 (A5B7 MAb) and the remaining immunogl bulin-derived parts of the HAM are derived from a human immunoglobulin. The HAMs may be chimeric humanised antibodies r CDR-grafted humanised antibodies and are preferably produced by recombinant DNA techniques. The HAMs are useful for *in vivo* diagnosis and therapy.

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+ DESIGNATIONS OF "SU"

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CDR grafted Anti-CEA antibodies and their production

FIELD OF THE INVENTION

The present invention relates to humanised antibody molecules (HAMs) having specificity for Carcinoembryonic Antigen (CEA) and to processes for their production using recombinant DNA technology.

The term "humanised antibody molecule" (HAM) is used to describe a molecule having an antigen binding site derived from an immunoglobulin from a non-human species, the remaining immunoglobulin-derived parts of the molecule being derived from a human immunoglobulin. The antigen binding site may comprise either complete variable regions fused onto human constant domains or only the complementarity determining regions (CDRs) grafted onto appropriate human framework regions in the variable domains. The abbreviation "MAb" is used to indicate a monoclonal antibody.

In the description, reference is made to publications by number, and these publications are listed in numerical order at the end of the description.

BACKGROUND OF THE INVENTION

Natural immunoglobulins have been known for many years, as have the various fragments thereof, such as the Fab, (Fab')₂ and Fc fragments, which can be derived by enzymatic cleavage. Natural immunoglobulins comprise a generally Y-shaped molecule having an antigen-binding site towards the outer end of each upper arm. The remainder of the structure, and particularly the stem of the Y, mediates the effect r functi ns ass ciat d with immunoglobulins.

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Natural immunoglobulins have been used in assay, diagnosis
and, to a more limit d ext nt, therapy.   However, such
uses, especially in therapy, have been hindered by th
polyclonal nature of natural immunoglobulins.   A
significant step towards the realisation of the potential
of immunoglobulins as therapeutic agents was the discovery
of procedures for the production of monoclonal antibodies
of defined specificity (1).   However, most MAbs are
produced by hybridomas which are fusions of rodent sple n
cells with rodent myeloma cells.   The resultant MAbs ar
therefore essentially rodent proteins.   There are v ry
few reports of the production of human MAbs.

Since most available MAbs are of rodent origin, they are
naturally antigenic in humans and thus can give rise to an
undesirable immune response termed the HAMA (Human
Anti-Mouse Antibody)_response.   Therefore, the use f
rodent MAbs as therapeutic agents in humans is inherently
limited by the fact that the human subject will mount an
immunological response to the MAb and will either remov
it entirely or at least reduce its effectiveness.

Therefore proposals have been made for making non-human
MAbs less antigenic in humans.   Such techniques can be
generically termed "humanisation" techniques.   These
techniques generally involve the use of recombinant DNA
technology to manipulate DNA sequences encoding the
polypeptide chains of the antibody molecule.

Early methods for humanising MAbs related to production of
chimeric antibodies in which an antigen binding site
comprising the complete variable domains of one antibody
are fused to constant domains derived from a second
antibody.   Methods for carrying out such chimerisation
procedures are described in EP0120694 (Celltech Limited);

EP0125023 (Gen ntech Inc.), EP-A-0171496 (Res. Dev. Corp. Japan), EP-A-0173494 (Stanford University), and EP-A-0194276 (Celltech Limited). The Celltech EP 0194276 application discloses a process for preparing an antibody molecule having the variable domains from a mouse MAb and the constant domains from a human immunoglobulin. It also describes the production of an antibody molecule comprising the variable domains of a mouse MAb, the CH1 and CL domains of a human immunoglobulin, and a non-immunoglobulin-derived protein in place of the Fc portion of the human immunoglobulin.

Subsequently a number of further patent applications have been published relating to chimeric antibodies, including tumour specific chimeric antibodies (e.g. WO 87/02671, Int. Gen. Eng. Inc.; EP 0256654, Centocor; EP 0266663, Int. Gen. Eng. Inc. & Oncogen; WO 89/00999, Int. Gen. Eng. Inc. and EP 0332424, Hybritech Inc.). The Genentech (EP0125023) and Hybritech (EP0332424) patent application relate to anti-carcinoembryonic antigen (anti-CEA) chimeric antibodies.

Such humanised chimeric antibodies, however, still contain a significant proportion of non-human amino acid sequence, i.e. the complete variable region domains. Thus such humanised antibodies may elicit some HAMA response, particularly if administered over a prolonged period [Begent et al (ref. 2)].

In an alternative approach, described in EP-A-02394000 (Winter), the complementarity determining regions (CDRs) of a mouse MAb have been grafted onto the framework regions of the variable domains of a human immunoglobulin by site directed mutagenesis using long oligonucleotides. Such CDR-grafted humanised antibodies are less likely to

give rise to a HAMA respons than humanised chimeric
antibodies in vi w f th low r pr porti n of n n-human
amino acid sequence which they contain.   There are 3 CDRs
(CDR1, CDR2 and CDR3) in each of the heavy and light chain
variable regions.

The earliest work on CDR-grafted humanised MAbs was
carried out on MAbs recognising synthetic antigens, such
as the NP or NIP antigens.   However, recently examples in
which a mouse MAb recognising lysozyme and a rat MAb
recognising an antigen on human T cells respectively were
humanised have been described by Verhoeyen et al (3) and
Riechmann et al (4).   The preparation of the CDR-graft d
antibody to the antigen on human T cells is also described
in WO 89/07452 (Medical Research Council).   More recently
Queen et al (5) have described the preparation of a
humanised CDR-grafted antibody that binds to the
interleukin 2 receptor.

It has been widely suggested that immunoglobulins, and in
particular MAbs, could potentially be very useful in th
diagnosis and treatment of cancer (6, 7).   There has
therefore been much activity in trying to produce
immunoglobulins or MAbs directed against tumour-specific
antigens.   So far, over one hundred MAbs directed against
a variety of human carcinomas have been used in various
aspects of tumour diagnosis or treatment (8).

There have been a number of papers published concerning
the production of chimeric monoclonal antibodies
recognising cell surface antigens.   For instance, Sahagan
et al (9) disclose a genetically engineered murine/human
chimeric antibody which retains specificity for a tumour-
associated antigen.   Also Nishimura et al (10) disclos a

recombinant murine/human chim ric mon cl nal antibody
specific for common acute lymphocytic leukemia antigen.

We have now prepared humanised antibodies to
carcinoembryonic antigen derived from the anti-CEA mouse
MAb A5B7 (11).

Our copending International Patent Application PCT/GB
90/02017 relates to the CDR-grafting of antibodies in
general and describes, among other things, that antib dies
having specificity for cancer markers such as CEA, e.g.
the A5B7 monoclonal antibody, have been successfully
CDR-grafted according to the procedure described therein.

SUMMARY OF THE INVENTION

According to the present invention, there is provided a
humanised antibody molecule (HAM) having specificity f r
carcinoembryonic antigen (CEA) and having an antigen
binding site wherein at least one of the complementarity
determining regions (CDRs) of the variable domain is
derived from the mouse monoclonal antibody A5B7 (A5B7 MAb)
and the remaining immunoglobulin-derived parts of the HAM
are derived from a human immunoglobulin.

GENERAL DESCRIPTION OF THE INVENTION

The HAM may comprise a chimeric humanised antibody or a
CDR-grafted humanised antibody.   When the HAM compris s a
CDR-grafted humanised antibody, the heavy and/or light
chain variable domains may comprise only one or two A5B7
derived CDRs;   though preferably all three heavy and light
chain CDRs are derived from A5B7.

The A5B7 MAb is a mouse MAb of the type IgG1-Kappa rais d
against purified CEA which had been denatured by h ating
to 85°C for 35 minut s.   The A5B7 MAb has been

ext nsively studied at Charing Cr ss Hospital, London, UK
(11).   Immunohistochemical studies have demonstrated that
the A5B7 MAb reacts with CEA producing tumours.   Its
distribution is within malignant glands in the cell
cytoplasm, at the cell surface and in necrotic debris.
However, it shows no significant cross-reactivity with a
wide spectrum of normal human tissues.   The molecular
cloning and sequencing of the A5B7 heavy and light chain
cDNAs is described hereinafter and the V_L and V_H cDNA and
predicted amino acid sequences are given in Figure 1.

Surprisingly it has been found that humanising the A5B7
MAb, in particular by CDR-grafting, does not substantially
adversely affect its binding activity, and this produces a
HAM which is extremely useful in both therapy and
diagnosis of certain carcinomas.

Preferably, the HAM of the present invention is produced
by recombinant DNA technology.

The HAM of the present invention may comprise: a compl te
antibody molecule, having full length heavy and light
chains;   a fragment thereof, such as the Fab, Fab′,
(Fab′)₂ or FV fragment;   a single chain antibody fragment,
e.g. a single chain FV;   a light chain or heavy chain
monomer or dimer;   including fragments or analogues of any
of these or any other molecule with the same specificity
as the A5B7 antibody.

The HAM of the present invention may have attached to it
an effector or reporter molecule.   For instance, th HAM
may have a macrocycle for chelating a heavy metal atom, or
a toxin such as ricin, attached to it by a covalent
bridging structure.   Alternatively, the procedures of
r combinant DNA technology may be used t produce a HAM in

which the Fc fragment, CH3 or CH4 domain f a complete
antib dy molecule has b en replaced by or has attached
thereto by peptide linkage a functional non-immunoglobulin
protein, such as an enzyme or toxin molecule.

The remaining non-A5B7, immunoglobulin derived parts of
the HAM may be derived from any suitable human
immunoglobulin.   For instance, when the HAM is a
CDR-grafted HAM, appropriate variable region framework
sequences may be used having regard to class/type of the
A5B7 donor antibody from which the antigen binding regions
are derived.   Preferably, the type of human framework
used is of the same/similar class/type as the donor
antibody (A5B7 is IgG1 Kappa).   Advantageously, the
framework is chosen to maximise/optimise homology with the
donor antibody sequence particularly at positions
spacially close or adjacent to the CDRs.   Examples of
human frameworks which may be used to construct
CDR-grafted HAMs are LAY, POM, TUR, TEI, KOL, NEWM, REI
and EU;   for instance KOL and NEWM for the heavy chain and
REI for the light chain and EU for both the heavy chain
and the light chain.   Preferably the LAY framework is
used as the human framework for both heavy and light chain
variable domains, in view of its high level of homology
with A5B7.

Also human constant region domains of the HAM may be
selected having regard to the proposed function of the
antibody, in particular the effector functions which may
be required.   For example, the constant region domains
may be human IgA, IgE, IgG or IgM domains.   In particular,
IgG human constant region domains may be used especially
of the IgG1 and IgG3 isotypes, when the HAM is intended
for therapeutic purposes and antibody effector functions
are requir d.   Alternatively, IgG2 and IgG4 isotypes may

be used wh n th HAM is intended for purposes for which
antibody eff ctor functi ns ar not requir d e.g. for
imaging, diagnostic or cytotoxic targeting purposes.

However, the remainder of the HAM need not comprise only
protein sequences from human immunoglobulins. For
instance, a gene may be constructed in which a DNA
sequence encoding part of a human immunoglobulin chain is
fused to a DNA sequence encoding the amino acid sequence
of a polypeptide effector or reporter molecule.

According to a second aspect of the present invention,
there is provided a process for producing the HAM of the
first aspect of the invention, which process comprises:

(a)   producing in an expression vector an operon having a
       DNA sequence which encodes an antibody heavy or light
       chain comprising a variable domain wherein at l ast
       one of the CDRs of the variable domain is derived
       from the A5B7 MAb and the remaining immunoglobulin-
       derived parts of the antibody chain are derived from
       a human immunoglobulin;

(b)   producing in an expression vector an operon having a
       DNA sequence which encodes a complementary antibody
       light or heavy chain comprising a variable domain
       wherein at least one of the CDRs of the variable
       domain is derived from the A5B7 MAb and the remaining
       immunoglobulin-derived parts of the antibody chain
       are derived from a human immunoglobulin;

(c)   transfecting a host cell with the or each vector;

and

(d)   culturing the transfected cell lin to produce th
      HAM.

The cell line may be transfected with two vectors, the
first vector containing an operon encoding a light
chain-derived polypeptide and the second vector containing
an operon encoding a heavy chain-derived polypeptide.
Preferably, the vectors are identical except in so far as
the coding sequences and selectable markers are concerned
so as to ensure as far as possible that each polypeptid
chain is equally expressed.

Alternatively, a single vector may be used, the vect r
including the sequences encoding both light chain- and
heavy chain-derived polypeptides.

Thus in further aspects the invention also includes DNA
sequences coding for the heavy and light chains, cloning
and expression vectors containing the DNA sequences, host
cells transformed with the DNA sequences and processes for
producing the heavy and/or light chains and antibody
molecules comprising expressing the DNA sequences in the
transformed host cells.

The general methods by which the vectors may be
constructed, transfection methods and culture methods are
well known per se.   Such methods are shown, for instance,
in references 12 and 13.

The DNA sequences which encode the A5B7 amino acid
sequence may be obtained by methods well known in the
art.   For example, the A5B7 coding sequences may be
obtained by genomic cloning, or cDNA cloning from the A5B7
hybridoma cell line.   Positive clones may be screened

using appropriate probes for the heavy and light chain
genes in questi n.   Also PCR cl ning may be used.

DNA coding for human immunoglobulin sequences may be
obtained in any appropriate way.   For example, DNA
sequences coding for preferred human acceptor frameworks
such as LAY, POM, KOL, REI, EU, TUR, TEI and NEWM, are
widely available to workers in the art.

The standard techniques of molecular biology may be used
to prepare DNA sequences coding for the CDR-grafted
products.   Desired DNA sequences may be synthesised
completely or in part using oligonucleotide synthesis
techniques.   Site-directed mutagenesis and polymerase
chain reaction (PCR) techniques may be used as
appropriate. For example, oligonucleotide directed
synthesis as described by Jones et al (14) may be used.
Also oligonucleotide directed mutagenesis of a
pre-existing variable region as, for example, described by
Verhoeyen et al (3) or Riechmann et al (4) may be used.
Also enzymatic filling in of gapped oligonucleotides using
T₄ DNA polymerase as, for example, described by Queen et
al (5) may be used.

Any suitable host cell/vector system may be used for
expression of the DNA sequences coding for the chimeric
CDR-grafted heavy and light chains.   Bacterial e.g.
E. coli, and other microbial systems may be used, in
particular for expression of antibody fragments, e.g. FV,
Fab and Fab' fragments, and single chain antibody
fragments e.g. single chain FVs.   Eucaryotic e.g.
mammalian host cell expression systems may be used f r
production of larger CDR-grafted antibody products,
including complete antibody molecules.   Suitable

mammalian host cells include CHO cells and myeloma or
hybridoma c ll lines.

The present invention also includes therapeutic and
diagnostic compositions containing the HAM of the
invention and uses of such compositions in therapy and
diagnosis.

Such therapeutic and diagnostic compositions typically
comprise a HAM according to the invention in combination
with a pharmaceutically acceptable excipient diluent or
carrier, e.g. for in vivo use. Therapeutic and
diagnostic uses typically comprise administering an
effective amount of a HAM according to the invention t a
human subject.

In the HAM of the first aspect of the invention and the
process of the second aspect of the invention the heavy
and light chain variable domains of the HAM may compris
either the entire variable domains of the A5B7 MAb or may
comprise framework regions of a human variable domain
having grafted thereon one, some or all of the CDRs of the
A5B7 MAb. Thus the HAM may comprise a chimeric humanised
antibody or a CDR-grafted humanised antibody.

When the HAM is a CDR-grafted humanised antibody, in
addition to the CDRs, specific variable region framework
residues may correspond to non-human, i.e. the A5B7 mouse,
residues. Preferably the CDR-grafted humanised
antibodies of the present invention include CDR-grafted
humanised antibodies as defined in our co-pending pat nt
application, International Patent Application PCT/GB
90/02017. The disclosure of PCT/GB 90/02017 is
incorporated herein by reference.

Pr ferably the CDRs of the light chain c rrespond to the
Kabat CDRs at CDR1 (positions 24-34) and CDR2 (positions
50-56) and to the structural loop residues (positions
91-96) or Kabat CDR residues (positions 89-97) in CDR3.
In addition the light chain may have mouse residues at on
or more of positions 1, 2 and/or 3, 46, 47, 49, 60, 70,
84, 85 and 87 and preferably has mouse residues at at
least positions 46 and 47.

In addition to the CDRs, the HAM heavy chain preferably
has mouse residues at positions 23 and/or 24 and 71 and/or
73. Additionally, the heavy chain may have mouse
residues at one, some or all of positions 48 and/or 49,
69, 76 and/or 78, 80, 88 and/or 91 and 6. Preferably
also, the CDRs of the heavy chain correspond to the Kabat
CDR at CDR2 (positions 50-65), the structural loop
residues at CDR3 (positions 95-100) and a composite of
both the Kabat and structural loop residues at CDR1
(positions 24-35); for example, when the human variabl
region framework used is KOL. Alternatively, the CDRs of
the heavy chain may comprise mouse residues at positions
26 to 35 for CDR1, positions 50 to 65 for CDR2 and
positions 94 to 100 for CDR3; for example, when the human
variable region framework used in EU. In addition EU has
a particularly idiosyncratic J region between residues 103
to 113 and it may be useful to include the murine amino
acids, or a consensus human J region or a suitable
combination of both at residues 103 to 108 inclusive.

In a particularly preferred embodiment LAY human variabl
region frameworks are used for both the CDR-grafted heavy
and light chains. In which case the light chain
preferably comprises mouse A5B7 residues at positions 1,
2, 3, 4, 46 and 71, and especially also at positions 21,
47 and 73, of the variable region framew rks. Similarly,

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th heavy chain pref rably comprises mouse A5B7 residu s at positions 1, 24, 48, 49, 72, 73, 76 and 93, and especially also at positions 82b and 86, of the variable region frameworks. Also when the LAY human variable region frameworks are used, the variable regions preferably comprises A5B7 mouse CDRs at residues 24 to 34 (CDR1) 50 to 56 (CDR2) and 89 to 97 (CDR3) for the light chain and at residues 26 to 35 (CDR1), 50 to 65 (CDR3) and 95 to 102 (CDR3) for the heavy chain.

The residue designations given above and elsewhere in the present application are numbered according to the Kabat numbering (15).

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention is now described, by way of illustration only, in the following examples which refer to the accompanying diagrams Figures 1 - 16, in which:-

Figure 1   shows the DNA sequences encoding the unprocessed variable regions of the A5B7 MAb obtained by sequencing cDNA clones together with the predicted amino acid sequence;

Figure 2   is a schematic diagram of the construction by restriction and ligation of the chimeric heavy chain gene;

Figure 3   is a schematic diagram of the construction by site-directed mutagenesis, restriction and ligation of the chimeric light chain gene;

Figure 4   shows an ELISA analysis of COS-cell transfectant supernatants. The level of antigen-binding capacity in the supernatant of

COS-cell transfectants was analysed as
described later.   Diluti n curves were
plotted out against the optical density of the
colour change;

Figure 5   shows plasmid diagrams for plasmids pBG7,
pBG11, pBG14, pHMC19, pHMC20 and pHMC21;

Figure 6   shows plasmid diagrams for plasmids pHMC29,
pHMC30 and pHMC31;

Figure 7   shows a SDS-PAGE gel of chimeric FAb' and
chimeric DFM products under both reducing and
non-reducing conditions;

Figure 8   shows the DNA and protein sequences for the
A5B7 grafted light chain, gL-1 variable regi n;

Figure 9   shows similar sequences for the A5B7 grafted
light chain gL-2, variable region;

Figure 10   shows similar sequences for the A5B7 grafted
heavy chain gH1, variable region;

Figure 11   shows similar sequences for the A5B7 grafted
heavy chain gH2, variable region.

Figure 12   shows plasmid diagrams for plasmids pMRR010,
pMRR014, pAL43, pAL44, pAL45 and pAL46;

Figure 13   is graphs showing the results of direct CEA
binding ELISAs on supernatants from transi nt
expression of chimeric/grafted hybrids and a
chimeric/chimeric standard;

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Figure 14   shows similar graphs for grafted/grafted
             transf ctions as well as the chimeric/chimeric
             standard;

Figure 15   shows plasmid diagrams of plasmids pMRR020,
             pAL45, pAL46, pAL49 and pAL50 indicating the
             derivation of the latter two plasmids;

Figure 16   shows plasmid diagrams of various plasmids
             indicating the derivation of plasmids pHMC43,
             pHMC44, pAL53 and pAL54;

Figure 17   shows plasmid diagrams for pACtac, pMMR28 and
             pMRR45;

Figure 18   shows graphs of CEA binding ELISAs on <u>E. coli</u>
             supernatants containing A5B7 chimeric Fab', and

Figure 19   shows similar graphs for A5B7 grafted Fab'.

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DETAILED DESCRIPTION OF SPECIFIC
EMBODIMENTS OF THE INVENTION

Example 1

Molecular cloning and sequencing of the A5B7 heavy and
light chain cDNAs

Polyadenylated RNA was isolated from the A5B7 hybridoma
cell line using the guanidinium isothiocyanate/lithium
chloride method (12).   Double stranded cDNA was
synthesised (16) and a cDNA library was constructed in
plasmid pSP64 (17) vector using EcoRI linkers.   Two
screening probes were synthesised, complementary to mous
immunoglobulin heavy and light chain constant regions.
The heavy chain probe was a 19 mer complementary to
residues 115-133 in the CH1 domain of the mouse γ 1
sequence (18).   The light chain probe was a 20 mer
complementary to residues 4658-4677 of the genomic mous
CK sequence (19).   The probes were radio-labelled at the
5' terminus with [γ ^{32}P] ATP using T4 polynucleotid
kinase (Amersham International) and used to screen the
cDNA library.

Clones which contained the complete leader, variable and
constant regions of both the heavy and light chains w re
isolated and designated as pBG1 and pBG2.   Nucleotid
sequence analysis was carried out according to the chain
termination procedure (20).

The 950 base pair EcoRI insert in pBG1 was fully
sequenced.   The EcoRI insert in pBG2 was shown to
comprise approximately 1700 base pairs by agarose gel
electrophoresis.   The variable domain and the 5' region
of the CH1 domain were sequenced, as was the 3' end of the
clon t confirm the pr s nc of the correct mous γ 1

t rminati n sequences.   Th DNA and predicted amino acid
sequenc s f r the unproc ssed variable regi ns of pBG1 and
pBG2 are shown in Figure 1.

With reference to Figure 1.   Panel A shows the sequence
coding for the V_L region and the predicted amino acid
sequence.   Panel B shows the sequence coding for th V_H
region together with the predicted amino acid sequence.
The putative sites of cleavage of the signal peptide are
indicated by arrows.   Examination of the derived amino
acid sequence revealed considerable homology with other
characterised immunoglobulin genes, and enabled the extent
of the leader, variable and constant domains to be
accurately determined.   In addition MAb A5B7 was confirmed
to be an IgG1 K antibody.

Example 2

Preparation and Testing of Chimeric Antibody Products

A. Construction of Chimeric Mouse-Human Heavy Chain Genes
The construction of vectors containing the human constant
region isotype series, pRB18(IgG1), pRB26(IgG2), pRB20
(IgG3) and pRB21 (IgG4) is described in published
International Patent Application WO 89/01783. The A5B7
V$_H$ DNA sequence was isolated as a EcoRI-BanI fragment and
was ligated to the following linking oligonucleotide to
give an EcoRI-HindIII V$_H$ fragment (Figure 2).

R1120   5' GCACCACTCTCACCGTGAGCTC
R1121   GTGAGAGTGGCACTCGAGTCGA5'

This fragment was ligated to the human HindIII-BamHI
containing fragments of the IgG1, 2, 3 & 4 genes cloned in
pAT153 to give pBG3, 4, 5 & 6.

The chimeric heavy chain genes were isolated as EcoRI-BamHI
fragments from the pAT IgG plasmids and cloned into pEE6
vector (21) cut with EcoRI & BclI, to give plasmids pBG
7,8,9 & 10. The pEE6 plasmid contains the strong
promoter/enhancer and transcriptional control element from
the human cytomegalovirus immediate early gene (hCMV-IE)
inserted into a unique HindIII site upstream of the EcoRI
site and is described in detail in published International
Patent Application WO 89/01036. In addition, an SV40
origin of replication is provided by the SV40 early
promoter fragment which drives as selectable marker gene,
a guanine phosphoribosyl transferase gene (gpt) inserted
into a unique BamHI site. The plasmid also contains an
ampicillin-resistance gene allowing selection and
propagation in bacterial hosts.

B. <u>Construction of the Chimeric Mouse-Human Light Chain Gene</u>

A HindIII restriction enzyme site was introduced into th mouse light chain cDNA clone, pBG1 by site-directed mutagenesis (23) using the following oligonucleotide:-

5'   TTTGATTTCAAGCTTGGTGC   3'

Introduction of the HindIII site was verified by DNA sequencing.   The A5B7 V_L sequence was isolated as an EcoRI/HindIII fragment.   It was combined with a HindIII/EcoRI fragment comprising the human Ck constant region and ligated into the unique EcoRI site of pEE6. (Figure 3).   The resulting plasmid pHMC19 also contain d the neomycin-resistant gene (neo) under the control of th SV40 early promoter.

C. <u>Transfections and ELISA Analysis of Antibody Production</u>

The four chimeric heavy chain expression constructs described above were transfected together with chimeric light chain into COS-1 cells (24) for transient e ression n of chimeric products.   The cells were left to in bat in DNA-DEAE dextran solution for six hours, then shocked for two minutes with 10% DMSO in HEPES-buffered saline.   The cells were washed and incubated in medium containing 10% foetal calf serum for 72 hours.

Following incubation at 37°C for 72 hours the cell supernatants were analysed by ELISA for heavy and light chain production and binding of antigen.

The medium (500 μl per 10^5 cells) was removed for ELISA analysis.

To quantify assembled antib dy pr ducti n Microtitre
plates were coated with 0.25 μg per well f sheep antibody
reactive against human specific epitopes on the heavy or
light chains.   Supernatants or lysates from transfected
COS cells were diluted 1:2 or 1:4 respectively in sample
conjugate buffer containing 0.1M Tris-HCl pH 7.0,
0.1M sodium chloride, 0.02% Tween 20 and 0.2% casein.
100 μl of each diluted sample were added to each well and
incubated for 1 hour at room temperature with gentle
agitation.   Following washing six times with wash buffer
(phosphate buffered saline containing 0.2% Tween 20, pH
7.2), 100 μl of 1:5000 dilution of standard horseradish
peroxidase – conjugated antibody reactive against human
specific epitopes were added per well.   The plates wer
incubated for 1 hour at room temperature, and then washed
six times with wash buffer.   100 μl of substrate buffer
containing 0.1 mg/ml tetramethylbenzidine (TMB), 0.1M
sodium citrate, pH 6.0 and 0.005% H_2O_2 were added to each
well to generate a colour change.   The reaction was
terminated after 2-3 minutes by adjusting the solution to
pH 1.0 with 1.5M sulphuric acid.   The optical density was
determined at 450nm for each well by measurement in a
Dynatech laboratories MR600 microplate reader.   Standard
curves were generated using known concentrations of the
appropriate human immunoglobulins.

Antigen binding assays were performed in an analogous
manner.   Microtitre plates were coated with 0.25 μg per
well of purified CEA.   Following washing six times in
wash buffer, samples from COS-cell transfections were
added as previously, and the same subsequent procedures
carried out, using goat anti-mouse or -human F(ab')$_2$
linked to HRP as the second antibody.

Assembly assays, which detect th presence of associat d
polypeptid chains, demonstrat d th formati n f
multimers containing at least one heavy and one light
chain when both genes were co-transfected. Antigen
binding analysis (see above) demonstrated that the
chimeric heavy and chimeric light chain co-transfections
generated an antibody molecule capable of recognising
antigen. The antigen binding ELISA data from one
experiment are presented in Figure 4. These experiments
demonstrate that chimerisation of the antibody molecule
does not have a significant effect on its antigen
recognition capability.

D. Immunoprecipitation of Antibody Molecules from
Biosynthetically Labelled COS-Cell Transfectants

Following transfection, COS cells were allowed to rec ver
for 24 hours in DMEM containing 10% foetal calf serum.
The medium was then replaced with methionine-free DMEM, to
which [^{35}S] methionine (NEN) had been added at 100 μCi/ml.
The cells were metabolically labelled for 48hours.
Analysis of the assembly and secretion of antibody
molecules was performed by immunoprecipitation using anti-
human F(ab')$_2$ bound to Protein A-Sepharose. Affinity-
purified rabbit antibodies against human IgG F(ab')$_2$ were
used for immunoprecipitations, following coupling to
Protein A - Sepharose. Secreted antibodies were analysed
on a SDS-10% PAGE system under reducing and non-reducing
conditions. The gel was treated with an autoradiography
enhancer, dried and exposed to Fuji RX film.

The antiserum immunoprecipitated proteins with an apparent
molecular weight of 55K and 28K, corresponding to the
heavy and light immunoglobulin chains respectively. A
comparison of immunoprecipitations analysed by reducing

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and non-reducing SDS-PAGE indicated that the heavy and
light chains wer assembled as the correct tetrameric
molecule.

<u>Example 3</u>

<u>Preparation and Comparison of Chimeric Whole Antibody and Fab' Products</u>

Stable cell lines expressing chimeric whole antibody and Fab' products were established and chimeric whole antibody, Fab', F(ab')2, and synthetically cross-linked DFM (Di Fab' Maleimide) products were prepared and test d.

First of all, however, it was necessary to construct a DNA sequence coding for the chimeric Fab' and vectors for expression of this sequence.

A. <u>Construction of Chimeric Mouse/Human Heavy Chain Gen and Vectors for Fab' Expression</u>

The plasmid containing the A5B7 chimeric heavy chain, IgG4, (pBG10) was restricted with BstEll and Bgl11. Th larger vector fragment containing the hCMV promoter and A5B7 V$_H$ plus the 5' part of CH1 domain was isolated. The plasmid pJA115 (described in International Patent Application WO 89/01974) was restricted with BstEll and Bgl11. A fragment containing the 3' end of CH1 plus th IgG4 hinge containing a cys to ala change was isolated and ligated into the pBG10 vector.

The resulting vector, pBG14, contains the A5B7 Fd' heavy chain IgG4 (cys to ala).

The Assay procedures used in this and subsquent Examples were as follows:

<u>Assembly ELISAs</u>

The ELISA for measuring yields of whole antibodies used microwell plates coated with goat F(ab')2 IgG Fc. Humanised IgG bound following incubati n with culture

sup rnatant samples was revealed with horseradish
peroxidase (HRP) conjugated murin anti-human kappa chain
antibody. Concentrations of chimeric or CDR-grafted
whole antibody in samples were interpolated from a
calibration curve generated from serial dilutions of
purified chimeric A5B7 IgG1.

The ELISA for measuring yields of Fab's used microwell
plates coated with murine anti-human IgG Fd. Following
incubation with samples bound humanised Fab' was revealed
as in the whole antibody assembly ELISA. Concentrations
of chimeric or CDR-grafted A5B7 Fab' in samples were
interpolated from a calibration curve generated from
serial dilutions of purified chimeric A5B7 Fab'.

CEA Binding Assays

The direct CEA binding ELISA used microwell plates coated
with CEA. Following incubation with serial dilutions of
culture supernatant samples bound IgG or fragments was
revealed as for the assembly ELISA. Binding versus
dilution curves were normalised against antibody
concentration as determined by the assembly assays.

The competition RIA for anti-CEA activity involved
competition of a series of ^{125}I-labelled murine or
chimeric A5B7 IgG1 with humanised IgG or fragments thereof
from culture supernatant samples for binding to CEA coated
beads. Binding activity was determined by measuring b ad
associated radioactivity. The assay was calibrated by
competition with standard preparations of chimeric or
murine A5B7 IgG1 and plotting % bound cpm versus antibody
concentration. Interpolated apparent A5B7 concentration
of unknowns was normalised by dividing by the assembly
assay result to give a specific activity. Finally
specific activity was expressed as % relative potency by

comparison to that obtain d from a p sitive control
chimeric A5B7 culture supernatant pr duced during the sam
experiment.

The relative potency of purified murine and chimeric A5B7
Fab' and F(ab')₂ fragments and murine IgG was investigated
using the competition RIA. In addition, the direct
binding ELISA was run in competition mode, by coincubation
of the test specimen with murine A5B7 IgG1, for
confirmation of relative potency.

Chimeric A5B7 Fab' was purified by ion-exchange
chromatography on DEAE-sepharose followed by hydrophobic
interaction chromatography on octyl-sepharose.
Cross-linking was carried out by the standard one pot
procedure using 1,6-bismaleimidohexane as cross-linker,
with a 2.2 fold excess of Fab' to cross-linker at
0.9mg/ml. Due to the small scale of the experiment
purification was carried out by HPLC gel filtration
(GF-250XL). This yielded an A5B7 chimeric DFM (Di Fab'
maleimide) product.

B. Development of CHO Cell Lines expressing Chimeric A5B7
IgG1 and Chimeric IgG4 Fab' delta cys
Two types of CHO cell line, amplifiable and
non-amplifiable, were developed expressing chimeric A5B7
IgG1 whole antibody and chimeric A5B7 IgG4 FAb' delta cys.
The chimeric A5B7 IgG1 whole antibody was used as a
standard for assay development and for comparison of
chimeric and grafted in biodistribution and therapy
studies in tumour-bearing mice.

Non-amplifiable cell lines
Non-amplifiable cell lines for chimeric whole antibody and
Fab' were first c nstructed. Although such cell lines

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are relatively low yielding, th y ar more r adily and
rapidly prepared and w re us d f r rapid generation of
material for development of procedures for purificati n
and for assays.   pHMC19 (see Example 2) is a plasmid
containing the chimeric A5B7 light chain 3' to the hCMV
promoter.   This plasmid was transfected into CHO-K1 cells
with selection for neomycin resistance and spot assays
performed on 30 transfectants with anti-human kappa
antibody to identify the best producing clones.   Assays
with the same antibody on representative cell lines
identified a stable cell line, designated HCN1.37,
secreting the chimeric A5B7 light chain with a specific
production rate of $1-2\mu g/ml/10^6$ cells.   The cell line was
retransfected with plasmids pBG7 and pBG14, as described
previously, these plasmids carrying the heavy chain genes
for chimeric A5B7 IgG1 and chimeric IgG4 Fab' delta cys
respectively, in the pEE6hCMV gpt vector (see Figure 5).
Cell lines yielding approximately 16mg/l chimeric IgG1
(designated HCN1.37/g1.1 and g1.7) and approximately 5mg/l
chimeric Fab' (designated HCN1.37/delta cys3) after
purification were identified among these retransfectants
by assembly and direct CEA binding assays.   These cell
lines were used to make test quantities of chimeric whole
antibody and Fab'.

Amplifiable Cell Lines

Cell lines capable of amplification by the GS vector
system and intended to give yields of chimeric A5B7 Fab'
delta cys suitable for eventual manufacture were then
constructed.   The ClaI-EcoRl fragment of pBG11 carrying
the hCMV promoter and the chimeric A5B7 light chain g ne
was first cloned between the ClaI and EcoRl sites of pEE12
(22) to give plasmid pHMC20 (see Figure 5).   Plasmid
pHMC30 (see Figure 6) carrying the genes for both the
chimeric light chain and chimeric IgG4 Fab' delta cys

heavy chain was constructed by ligating the large
Na 1-Fsp1 fragment of pHMC20 (carrying the light chain
gene) to the EcoRV-Fsp1 fragment of pHMC28, a derivative
of pBG14 in which the gpt gene has been removed from the
BamHI site.   pHMC30 is a double gene plasmid suitable for
development of an amplifiable cell line, using the mouse
myeloma cell line NSO as host cell line, expressing
chimeric A5B7 Fab' since it contains the GS cDNA for
selection of transfectants.   Selection and amplification
of transfectants in CHO cells requires expression of the
GS minigene rather than the cDNA.   A double gene plasmid
suitable for development of an amplifiable CHO cell line
was constructed by ligating the Mlu1-Fsp1 fragment of
pHMC30, carrying both the chimeric light and heavy chain
expression units, with the GS minigene containing fragment
of pEE14 (25).   The resulting plasmid, designated pHMC31
(see Figure 6), was transfected into CHO-K1 cells with
selection on 25μM MSX.   Specific production rates were
determined on these transfectants, and seven chosen for
amplification.   The specific production rates for these
seven transfectants before amplification were as follows
(in units of $\mu g/10^6$ cells/24hrs):   HC3.36, 1.3;   HC3.21,
0.65;   HC3.33, 0.65;   HC5.19, 0.14;   HC5.24, 3.4;   HC5.33,
3.7;   HC5.39, 0.35.   Selection for cells potentially with
high copy number of the inserted plasmid sequences was
achieved by increasing the MSX concentration to between
100 and 1000 μM, screening for surviving cell lines and
measuring specific production rates.   Specific copy
number estimates were not done.

C. <u>Purification of Chimeric A5B7 IgG1</u>
Chimeric IgG1 was purified by a modification of the
procedure described for B72.3 by Colcher <u>et al</u> (26).   CHO
cell supernatants were concentrated by spiral cartridge
ultrafiltrati n, then purified by affinity chromatography

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on pr tein A Sepharose, with elution at pH3.   Reducing
and non- reducing SDS-PAGE showed the purifi d antibody to
be fully assembled, with a purity of > 95%.

D. Purification and Cross-linking of Chimeric A5B7 Fab'
Chimeric A5B7 Fab' was purified by ion-exchange
chromotography on DEAE-Sepharose following by hydrophobic
interaction chromotography on octyl-Sepharose.   CHO cell
culture supernatant containing A5B7 Fab' was concentrated
ten fold by ultrafiltration and diluted to the original
volume with 10mM tris pH 7.5 to reduce the conductivity to
< 4 mS.   This material was then applied to a column of
DEAE-Sepharose fast flow pre-equilibrated with 10mM tris
pH 7.5, and the flow through which contains the Fab'
collected.   The flow through from the DEAE-Sepharose
column was then concentrated by ultrafiltration and mad
2M in ammonium sulphate.   Any precipitate was removed by
centrifugation and the sample then applied to a column of
octyl-Sepharose pre-equilibrated with 10mM tris pH 7.5
containing 2M ammonium sulphate.   The Fab' bound to th
column and was washed with equilibration buffer and elut d
by decreasing the ammonium sulphate concentration to 1M.
The elute was then dialysed into 100mM sodium
acetate/citrate pH6 and concentrated by ultrafiltration.

The purified Fab' was cross-linked by firstly generating a
free thiol at the hinge followed by cross-linking with 1,6-
bismaleimidohexane.   Partial reduction to generate a free
thiol was achieved by incubation of the Fab' with 4.5mM
β-mercaptoethylamine for 30 minutes at 37°C.   The
reducing agent was then removed by desalting on a column
of Sephadex G-25 and the reduced Fab' immediately cross-
linked by incubation with 1,6-bismaleimidohexane at a
molar ratio of 1:2.2 bismaleimidohexane:Fab' with a Fab'
conc ntration of 0.9 mg/ml.   After overnight incubation

at 37°C the cr ss-linked material was purified by HPLC gel
filtration using a DuPont Zorbax GF-250XL column in 0.2M
phosphate pH 7.0. · The cross-linking yield was
approximately 58%.

The purified Fab' and cross-linked di-Fab (DFM) were
analysed by SDS-PAGE under both reducing and non-reducing
conditions (Figure 7). The purified Fab' ran as expected
with a molecular weight of approximately 50 KDa under
non-reducing conditions which reduced to Fd' and light
chains at 25KDa. The purified cross-linked di-Fab
revealed the expected molecular weight for the non-reduc d
dimer of 100 KDa which reduced to cross-linked Fd' at
approximately 50 KDa and light chain at approximately 25
KDa.

E. Antigen Binding Activity of Chimeric A5B7 IgG1, Fab'
and DFM

The relative potencies of murine and chimeric IgGs and
fragments were measured by the competition RIA. The
results are given in Table 1. All the bivalent speci s -
including the chimeric DFM - gave potency equivalent to
that of murine A5B7 IgG. The murine Fab' rendered
monovalent by alkylation, showed a tenfold reduced
potency, as would be predicted by avidity
considerations. The monovalent chimeric Fab', however,
displayed an intermediate potency. Measurements of
binding activity on the same samples using the direct
binding ELISA in competition mode gave very similar
results, with the chimeric Fab' again showing an
intermediate potency. Since the chimeric Fab' was not
alkylated its increased binding activity compared to the
murine Fab' is likely to be due to some degree of antigen
induced dimerisation.

Table 1

Relative potencies of various A5B7 constructs by
competition RIA

Construct	% Relative Potency
Chimeric IgG	100
Murine IgG	93 + 23
Chimeric DFM	104 + 28
Murine F(ab)$_2$	97 + 22
Chimeric Fab'	20 + 15
Murine Fab'	8 + 5

<u>Example 4</u>

Humanised, CDR-grafted versions of A5B7 were also prepared.

<u>Construction and Expression of CDR-grafted A5B7 Genes</u>

Figure 1 shows the DNA and amino acid sequences of the V_L
and V_H domains for A5B7.   CDR-grafted V_H and V_L domains
were designed substantially as described in Internati nal
Patent Application No. PCT/GB 90/02017.   The amino acid
sequence of A5B7 V_H shows considerable homology to the
consensus sequence of the human V_H III subgroup, as
defined by Kabat <u>et al</u>, (15), while the V_L sequence shows
considerable homology to those of the human VII and III
subgroups.   The human framework sequences available
within these subgroups are: LAY(V_H:V_L), POM(V_H:V_L), KOL
(V_H):REI(V_L), KOL(V_H):   EU(V_L), TUR(V_H):   REI(V_L),
TUR(V_H):Eu(V_L), TEI(V_H):REI(V_L), TEI(V_H):EU(V_L).
Of these LAY was chosen as the human framework because it
has the highest homology to A5B7 and also the potential
advantage of matched V_H and V_L chains.   CDR sequences and
other residues potentially important for antigen binding
were identified as described in International Patent
Application No. PCT/GB 90/02017, and for each V region two
constructs were assembled.   The first constructs, gL1 and
gH1, contain murine sequences in the CDRs and at other
positions predicted to be important for antigen binding
and at which human and A5B7 sequences differ.   The gL1
light chain has murine CDRs at residues 24-34 (CDR1),
50-56 (CDR2) and 89-97 (CDR3) and additional murine
residues within the frameworks at residues 1, 2, 3, 4, 46
and 71.   The gH1 heavy chain has murine CDRs at residues
26-35 (CDR1), 50-65 (CDR2) and 95-102 (CDR3) and
additional murine residues within the frameworks at
residues 1, 48, 49, 72, 73, 76 and 93.

Th second constructs, gL2 and gH2, are more conservativ ,
containing the murine sequ nces present in th first
constructs together with three extra murine residues for
V_L and two for V_H.   For gL2 these residues (positions 21,
47 and 73 on the Kabat numbering system) are potentially
involved in packing of the domain.   For gH2 the extra
murine residues are at positions (82b and 86) where LAY
has amino acids which are unusual for human V_H sequenc s
and where A5B7 has residues of more common occurrence in
human V_Hs.

Figures 8, 9, 10 and 11 show the DNA and amino acid
sequences of gL1, gL2, gH1 and gH2 respectively.   In
these Figures single letter underlining in the amino acid
sequences indicate a residue in the framework region which
has been changed to the corresponding murine residue.
Also in these Figures solid single line underlining of the
amino acid sequences indicates the CDR residues.   These
DNA sequences were assembled from oligonucleotides
(indicated by double line underlining in the respectiv
Figure) by the PCR overlap-assembly procedure, using a
Polymerase Chain Reaction (PCR) procedure, using
oligonucleotides of approximately 80 bases alternating on
the sense and anti-sense strands.   The oligonucleotides
overlapped by 20 bases, such that annealing led to th
formation of partially double stranded molecules.   The
gaps were filled in by Taq polymerase and the double
stranded material amplified by PCR using short
oligonucleotides corresponding to the sequence of the 5'
end of each strand as amplifiers.   The amplified
fragments were digested with appropriate restriction
enzymes to expose the restriction sites for cloning.

Amplified fragments of the appropriate size were digested
with HindIII and ApaI for V_H, and BstBI and SplI for V_L to

expose the cloning sites.   Th V$_H$ fragments were cloned
into the expr ssion v ctor pMRR014 and the V$_L$ fragments
into pMRR010.   pMRR010 and pMRR014 are hCMV expression
vectors designed to accept humanised V regions so as to be
readily expressed transiently in CHO or COS cells and then
readily to be reconstructed to give a single vector
capable of stable expression and gene amplification in NSO
cells.   This gave plasmids pAL43 (for gL1), pAL44 (gL2),
pAL45 (gH1) and pAL46 (gH2) as shown in Figure 12.   These
plasmids contain the full length CDR-grafted antibody
genes (IgG1 for heavy chain, kappa for light chain) in a
configuration suitable for expression in CHO cells.
Clones containing the correct sequence were identified by
DNA sequencing.

In order to assess the CEA binding activity of the grafted
chains co-expression, experiments in a transient CHO cell
system were first performed with chimeric partners.   Thus
pAL43 and pAL44 were co-transfected into the CHO L761 h
cell line (27) together with the chimeric A5B7 heavy chain
expression plasmid pBG7, while pAL45 and pAL46 were
co-transfected with the light chain expression plasmid
pBG11.   Co-transfections of pBG7 and pHMC19 were also
performed to provide a chimeric/chimeric standard against
which to compare activity of the chimeric/grafted
hybrids.   Figure 13 shows the results of direct CEA
binding ELISAs on the crude supernatants resulting from
these transfections, and indicates that all the
grafted/chimeric hybrids show binding activity similar to
that of the chimeric/chimeric standard.   Considerable
variation was observed between the various hybrids in the
yields of antibody.   This variation was also very
apparent when grafted/grafted co-transfections were
performed.   Indeed, the yields observed for gL1/gH1 and
gL2/gH1 were too l w to permit reliable estimat s of CEA

binding activity.   Both the gL1/gH2 and gL2/gH2
combinations, however, bound CEA at approximat ly 60% as
well as the double chimeric antibody in the direct binding
assay (see Figure 14).   Competition RIAs were also
performed on the crude CHO cell supernatants for gL1/gH2
and gL2/gH2.   The results, shown in Table 2, show that
these grafted variants displayed approximately 42% and 47%
respectively of the potency of the chimeric in these more
stringent assays, with gL2/gH2 showing slightly greater
potency than gL1/gH2.

Table 2

Estimates of Anti-CEA Activity in Transfected CHO Cell Supernatants for Grafted Variants of A5B7 by Competition RIA

Undiluted

	Specific activity	relative potency
Construct		
Chimeric A5B7	1.17	100±6
Grafted A5B7 gL1/gH2	0.49	42±4
Grafted A5B7 gL2/gH2	0.55	47±3

Example 5

Similarly CDR-grafted A5B7 Fab' genes were constructed and expressed.

A. Construction of CDR-grafted A5B7 Fab' Genes
pMRR020 is a pEE6 gpt expression plasmid with restriction sites such that coding sequences of the human IgG4 CH1 and delta cys (i.e. single cysteine variant) hinge domains (see Figure 15). A fragment containing these two domains was isolated by performing a PCR reaction on pMRR020 using oligonucleotide R1053 as the forward primer and R2371 as the back primer as shown below.

R1053   5' GTCGACAGACTAACAGACTGTTCC   3'

R2371   5' ATGATCAATGAATTCATCATGGGGCTGATGGGCACGGGGGACCATATTT GGACTC 3'

Use of the latter primer results also in the removal of the inconvenient Apa1 site in the hinge coding region. The PCR reaction produced a fragment of 320bp, which was cloned into pAL45 and pAL46 to give pAL49 and pAL50 respectively (Figure 15) carrying the full length CDR-grafted Fd delta cys genes. The CH1 and hinge domains and the cloning junction regions were sequenced to confirm the absence of secondary mutations.

B. Development of CHO Cell Lines Expressing CDR-grafted A5B7 IgG1 Whole Antibody and CDR-grafted A5B7 IgG4 Fab' delta cys genes
To make stable cell lines expressing CDR-grafted IgG1 whole antibody and CDR-grafted Fab' the gL1 and gL2 g nes were first isolated as Cla1-EcoR1 fragments from pAL43 and pAL44 and cloned into the vect r pMRR017, a derivative of

pEE14 with a useful poly-link r ins rted at the BamHI sit
t give plasmids pHMC36 and pHMC37 r spectively (s e
Figure 16). The gH1(IgG1), gH2(IgG1), gH1(Fd delta cys)
and gH2(Fd delta cys) genes were isolated as Hind111-BamH1
fragments from pAL45, 46, 49 and 50 respectively and
cloned along with an EcoR1-Hind111 fragment carrying th
SV40 polyA and hCMV promoter between the EcoR1 and BamH1
sites of pHMC36 to give plasmids pHMC43 (gL1-gH1),
pHMC44(gL1-gH2), pAL53 (gL1-gH1Fd delta cys) and pAL54
(gL1-gH2Fd delta cys). These GS double gene expression
plasmids (see Figure 16) were transfected into CHO-K1
cells to give CDR-grafted IgG1 whole antibody and
CDR-grafted Fab' producing cell lines substantially as
described in previous Examples.

Example 6

Production of A5B7 antibody fragments in E. coli

Chimeric and CDR-grafted A5B7 Fab' fragments (the gL1gH2
CDR-grafted variant was used) were also expressed in an
E. coli secretion system, this being the preferred
expression host for large scale production of antibody
fragments.

For expression/secretion in E. coli the natural signal
sequences of the A5B7 heavy and light chains were first
replaced with the signal sequence of the E. coli outer
membrane protein ompA (Movva et al, 28). A 92 base pair
fragment encoding the ompA signal sequence and including
the ompA translation initiation region was assembled from
oligonucleotides and cloned into the phagemid vector pSK⁺
(from Stratagene Cloning Systems) between the XhoI and
HindIII sites. The DNA and amino acid sequence of the 92
base pair fragment were as follows:

```
                        metlyslysthralailealailealavalala
TCGAGTTCTAGATAACGAGGCGTAAAAAATGAAAAAGACAGCTATCGCGATTGCAGTGGCA
    CAAGATCTATTGCTCCGCATTTTTTACTTTTTTCTGTCGATAGCGCTAACGTCACCGT
```

```
leualaglyphealathrvalalalaglnala
CTGGCTGGTTTCGCTACCGTAGCGCA
GACCGACCAAAGCGATGGCATCGCGTTCGA
```

A clone shown by DNA sequencing to carry the above
sequence in pSK⁺ was designated pSKompA.

For the chimeric A5B7 light chain a 650 base pair
SacI-EcoRI fragment encoding most of V_L and all of C kappa
was isolated from pHMC19. A pr cise fusion of the

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chimeric Light chain to the ompA signal s quence was mad
by ligating this Sacl-Ec Rl fragment into pSKompA digested
with Hindlll and EcoRl together with a 54 base pair
Hindlll-Sacl fragment assembled from oligonucleotides and
comprising the DNA sequence encoding the 3' region of the
ompA signal sequence and the 5' region of A5B7 V_L.   The
sequence of the 51 base pair fragment was as follows:

```
5'   AGCTCAAACTGTTCTCTCCCAGTCTCCAGCAATCCTGTCTGCATCTC          3'
3'       GTTTGACAAGAGAGGGTCAGAGGTCGTTAGGACAGACGTAGAGGTCC    5'
```

A clone containing the correct sequence was identified by
DNA sequencing and designated pSKompA-cLc.

For the chimeric A5B7 heavy chain a 580 base pair
Avall-EcoRl fragment encoding most of V_H and all of the
CH1 and (delta cys) hinge domains was isolated from
pBG14.   A precise fusion of the chimeric heavy chain to
the ompA signal sequence was made by ligating the
Avall-EcoRl fragment into pSKompA digested with Hindlll
and EcoRl together with a 120 base pair fragment assembled
from oligonucleotides and comprising the DNA sequence
encoding the 3' end of the ompA signal sequence and the 5'
region of V_H.   The sequence of the 120 base pair fragment
was as follows:

```
5' AGCTGAGGTGAAGCTTGTGGAGTCTGGAGGAGGGTTGGTACAGCCTGGGGGTTCTCTGA
3'     CTCCACTTCGAACACCTCAGACCTCCTCCGAACCATGTCGGACCCCCAAGAGACT
```

```
GACTCTCCTGTGCAACTTCTGGGTTCACCTTCACTGATTACTACATGAACTGG          3'
CTGAGAGGACACGTTGAAGACCCAAGTGGAAGTGACTAATGATGTACTTGACCCAGG 5'
```

A clone containing the correct sequence was identified by
DNA sequencing and designated pSKompA-cFd.

For expression of the chimeric Fab' the ompA-cLc fusion
was th n removed from pSKompA-cLc on a XhoI-EcoRl fragment
and cloned into the expression vector pACtac digested with
SalI and partially with EcoRl (see Figure 17).   pACtac
was constructed by replacing the Amp^r selectable marker
and pUC18-derived replication functions of the expression
plasmid, pTTQ9 (Amersham International) with the Cm^r
selectable marker and replication functions of pACYC184
(Chang & Cohen, 1978, J. Bacteriol. 134: 1141-1156).
(Partial EcoRl digestion was required because pACtac
contains a second EcoRl site, in the Cm^r gene.)   A
plasmid with ompA-cLc inserted adjacent to the tac
promoter was identified by restriction mapping and DNA
sequencing and designated pMRR024.

The ompA-cFd fragment was removed from pSKompA-cFd as a
XhoI-SmaI fragment and cloned into pSP73 (Promega
Corporation) digested with SalI and PvuII, to give a
plasmid designated pMRR027.   The ompA-cFd fusion was then
removed from pMRR027 as an EcoRl fragment, which was
cloned into pMRR024 partially digested with EcoRl.   A
clone carrying ompA-cFd oriented for transcription from
the tac promoter (along with ompA-cLc) was identified by
restriction mapping and DNA sequencing and designated
pMRR028.

pMRR028 was transformed into E. coli strain W3110 (ATCC
strain 27325).   Strain W3110 (pMRR028) was grown in a
1.5L fermenter in medium containing chloramphenicol to
maintain selection for retention of the plasmid.   At a
culture O.D 600nm of 10 expression of the A5B7 Fab' genes
from the tac promoter was induced by adding the inducer
IPTG to a final concentration of 1mM.   Direct CEA binding
assays were performed on crude culture supernatant samples
taken from this culture at time points 0, 3, 4, 5, 6, 7

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and 8 h urs af: r inducti n.   Th results of these
assays, together with that of a chimeric A5B7 Fab'
standard made and purified from mammalian cells are shown
in Figure 18.   They demonstrate the accumulation of
active chimeric A5B7 Fab' in the E. coli culture medium.
SDS-PAGE analysis on the same crude culture medium samples
revealed proteins with mobility of the size expected f r
Fab' heavy and light chains.   N-terminal protein
sequencing on these proteins revealed the N-terminal amino
acid sequences of mature A5B7 heavy and light chains,
showing that the ompA signal sequence had been accurately
cleaved from both.   Estimates (by OD 280nM measurements
and by SDS-PAGE) for the chimeric Fab' in the E. coli
culture medium were performed: the results suggested
yields in excess of 30mg/L after purification.

For the CDR-grafted A5B7 Fab' light chain a 620 base pair
Hph1-EcoR1 fragment encoding most of V_L for the gL1
variant and all of C kappa was isolated from pAL43.   A
precise fusion of the grafted light chain to the ompA
signal sequence was made by ligating the Hph-EcoR1
fragment into pSKompA digested with Hind111 and EcoR1
together with a 62 base pair fragment assembled from
oligonucleotides and encoding the 3' end of the ompA
signal sequence and the 5' end of the CDR-grafted V_L.
The sequence of the synthetic fragment was as follows:

AGCTCAGACTGTACTCACTCAGAGTCCAAGTAGTCTCAGTGTAAGTGTAGGTGATAGGGTAA
        GTCTGACATGAGTGAGTCTCAGGTTCATCAGAGTCACATTCACATCCACTATCCCAT

A clone carrying the correct sequence was identified by
DNA sequencing and designated pMRR034.

For the CDR-grafted A5B7 Fab' heavy chain gene a 720 base
pair Pvu11-Ec R1 fragment encoding m st of V_H f r th gH2

variant and all of CH1 and the (delta cys) hinge domains
was isolated from pAL50.   A precis fusion of this
grafted H chain to the ompA signal sequence was made by
ligating this Pvull-EcoR1 fragment into pSKompA digested
with Hindlll and EcoR1 together with a very short fragment
assembled from oligonucleotides and encoding the 3' end of
the ompA signal and the 5' end of the CDR-grafted V_H.
The sequence of the short adaptor fragment was as follows:

          5' AGCTGAGGTGCAG 3'
               3' CTCCACGTC 5'

A clone containing the correct sequence was identified by
DNA sequencing and designated pMRR037.

For expression of CDR-grafted Fab' the ompA-gL1 fusi n was
taken from pMRR034 as a Xhol-EcoR1 fragment and clon d
into pACtac digested with Sall and partially with EcoR1.
A clone carrying ompA-gL1 adjacent to the tac promoter was
identified by restriction mapping and DNA sequencing and
designated pmRR038.   The ompA-gH2 fusion was then taken
from pMRR037 as a Xhol-Smal fragment and cloned into pSP73
doubly digested with Pvull and EcoR1 to give a plasmid
designated pMRR041.   The ompA-gH2 fusion was then removed
from pMRR041 as an EcoR1 fragment and cloned into pMRR038
partially digested with EcoR1.   A clone carrying the
ompA-gH2 fusion oriented for transcription from the tac
promoter (along with ompA-gL1) was identified by
restriction mapping and DNA sequencing and designated
pMRR045.

pMRR045 was transformed into E. coli strain W3110 and the
W3110 (pMRR045) strain resulting was grown in a 1.5L
fermenter.   Expression of the CDR-grafted Fab' genes was
induced as described ab ve for the chimeric Fab' genes.

Crude culture supernatant sampl s fr m this culture at
time p ints 1, 4, 5, 6, 11 and 22 h urs after induction
were used in direct CEA binding assays and yield
estimates.   The results of the CEA binding assays are
given in Figure 19 and show the accumulation of material
active in antigen binding.   SDS-PAGE analysis of these
supernatant samples demonstrated the presence of proteins
of the size expected, and suggested yields in excess of
30mg/L.

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CLAIMS

1. A humanised antibody molecul (HAM) having
   specificity for carcinoembryonic antigen and having
   an antigen binding site wherein at least one of the
   complementarity determining regions (CDRs) of the
   variable domains is derived from the mouse monoclonal
   antibody A5B7 (A5B7 MAb) and the remaining
   immunoglobulin-derived parts of the HAM are derived
   from a human immunoglobulin.

2. A HAM according to Claim 1 which is a chimeric
   humanised antibody.

3. A HAM according to Claim 1 which is a CDR-graft d
   humanised antibody.

4. A HAM according to any one of Claims 1-3 when
   produced by recombinant DNA technology.

5. A HAM according to any one of claims 1-4, which
   comprises a complete antibody molecule, or a Fab,
   Fab', (Fab')2 or Fv fragment, or a single chain
   antibody fragment.

6. A HAM according to any one of claims 1-5, wherein an
   effector or reporter molecule is attached thereto.

7. A CDR-grafted HAM according to any one of Claims 3-6
   comprising LAY, POM, TUR, TEI, KOL, NEWM, REI or EU
   variable region framework sequences for the heavy
   and/or light chains.

8. A CDR-grafted HAM according to Claim 7 comprising LAY
   variable region framework sequences for both the
   h avy and light chains.

9.   A CDR-grafted HAM acc r _ng to any one of Claims 3-8
      having A5B7 CDRs at positi ns 24 to 34 (CDR1), 50 to
      56 (CDR2) and 92 to 96 or 89 to 97 (CDR3) of th
      light chain variable region.

10.   A CDR-grafted HAM according to any one of Claims 3-9
      comprising A5B7 residues at one or more of positions
      1, 2 and/or 3, 46, 47, 49, 60, 70, 84, 85 and 87,
      preferably at at least positions 46 and 47, of the
      light chain variable region.

11.   A CDR-grafted HAM according to any one of Claims 3-10
      having A5B7 CDRs at positions 24 to 35 or 26 to 35
      (CDR1), 50 to 65 (CDR2) and 95 to 100 or 94 to 100
      (CDR3) of the heavy chain variable region.

12.   A CDR-grafted HAM according to any one of Claims 3-11
      having A5B7 residues at any one or more of positi ns
      23 and/or 24, 71 and/or 73, 48 and/or 49, 69, 76
      and/or 78, 80, 88 and/or 91 and 6 of the heavy chain
      variable region.

13.   A CDR-grafted HAM according to Claim 8, comprising
      A5B7 residues at positions 1, 2, 3, 4, 46 and 71, and
      especially also at positions 21, 47 and 73, of the
      light chain, and at positions 1, 24, 48, 49, 72, 73,
      76 and 93, and especially also at positions 82b and
      86, of the heavy chain.

14.   A CDR-grafted HAM according to Claim 13, comprising
      A5B7 CDRs at positions 24 to 34 (CDR1), 50 to 56
      (CDR2) and 89 to 97 (CDR3) of the light chain, and at
      positions 26 to 35 (CDR1) 50 to 65 (CDR2) and 95 to
      102 (CDR3) of the heavy chain.

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15. A therapeutic or diagnostic composition comprising a
    HAM according to any on of the preceding claims in
    combination with a pharmaceutically acceptable
    excipient, diluent or carrier.

16. A therapeutic or diagnostic method comprising
    administering an effective amount of a therapeutic or
    diagnostic composition according to Claim 15 to a
    human subject.

17. A process for producing a HAM according to any one of
    claims 1 to 14, which process comprises:-

    (a) producing in an expression vector an operon
         having a DNA sequence which encodes an antibody
         heavy or light chain comprising a variable
         domain wherein at least one of the CDRs of th
         variable domain is derived from the A5B7 MAb and
         the remaining immunoglobulin-derived parts f
         the antibody chain are derived from a human
         immunoglobulin;
    (b) producing in an expression vector an operon
         having a DNA sequence which encodes a
         complementary antibody light or heavy chain
         comprising a variable domain wherein at least
         one of the CDRs of the variable domain is
         derived from the A5B7 MAb and the remaining
         immunoglobulin-derived parts of the antibody
         chain are derived from a human immunoglobulin.
    (c) transfecting a host cell with the or each
         vector; and
    (d) culturing the transfected cell line to produce
         the HAM.

18. A pr cess according to Claim 17, wherein the heavy
    and light chain encoding sequ nces are present on th
    same vector.

19. A process according to Claim 17, wherein the heavy
    and light chain encoding sequences are present on
    separate vectors.

20. A process according to any one Claims 17-19 for the
    production of an antibody fragment in which the host
    cell is a bacterial host cell.

21. A process according to any one of Claims 17-19 in
    which the host cell is a mammalian host cell.

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Fig. 1

A5B7: light chain sequence
DNA and protein sequence of the VI domain

```
ATGGATTTTCAAGTGCAGATTTTCAGCTTCCTGCTAATCAGTGCTTCAGTCATAATGTCC
 M  D  F  Q  V  Q  I  F  S  F  L  L  I  S  A  S  V  I  M  S

AGAGGACAAACTGTTCTCTCCCAGTCTCCAGCAATCCTGTCTGCATCTCCAGGGGAGAAG
 R  G  Q  T  V  L  S  Q  S  P  A  I  L  S  A  S  P  G  E  K

GTCACAATGACTTGCAGGGCCAGCTCAAGTGTAACTTACATTCACTGGTACCAGCAGAAG
 V  T  M  T  C  R  A  S  S  S  V  T  Y  I  H  W  Y  Q  Q  K

CCAGGATCCTCCCCCAAATCCTGGATTTATGCCACATCCAACCTGGCTTCTGGAGTCCCT
 P  G  S  S  P  K  S  W  I  Y  A  T  S  N  L  A  S  G  V  P

GCTCGCTTCAGTGGCAGTGGGTCTGGGACCTCTTACTCTCTCACAATCAGCAGAGTGGAG
 A  R  F  S  G  S  G  S  G  T  S  Y  S  L  T  I  S  R  V  E

GCTGAAGATGCTGCCACTTATTACTGCCAACATTGGAGTAGTAAACCACCGACGTTCGG
 A  E  D  A  A  T  Y  Y  C  Q  H  W  S  S  K  P  P  T  F  G

GGAGGCACCAAGCTGGAAATCAAACGG
 G  G  T  K  L  E  I  K  R
```

A5B7: heavy chain sequence
DNA and protein sequence of the Vh domain

```
ATGAAGTTGTGGCTGAACTGGATTTTCCTTGTAACACTTTTAAATGGTATCCAGTGTGAG
 M  K  L  W  L  N  W  I  F  L  V  T  L  L  N  G  I  Q  C  E

GTGAAGCTGGTGGAGTCTGGAGGAGGCTTGGTACAGCCTGGGGGTTCTCTGAGACTCTCC
 V  K  L  V  E  S  G  G  G  L  V  Q  P  G  G  S  L  R  L  S

TGTGCAACTTCTGGGTTCACCTTCACTGATTACTACATGAACTGGGTCCGCCAGCCTCCA
 C  A  T  S  G  F  T  F  T  D  Y  Y  M  N  W  V  R  Q  P  P

GGAAAGGCACTTGAGTGGTTGGGTTTTATTGGAAACAAAGCTAATGGTTACACAACAGAG
 G  K  A  L  E  W  L  G  F  I  G  N  K  A  N  G  Y  T  T  E

TACAGTGCATCTGTGAAGGGTCGGTTCACCATCTCCAGAGATAAATCCCAAAGCATCCTC
 Y  S  A  S  V  K  G  R  F  T  I  S  R  D  K  S  Q  S  I  L

TATCTTCAAATGAACACCCTGAGAGCTGAGGACAGTGCCACTTATTACTGTACAAGAGAT
 Y  L  Q  M  N  T  L  R  A  E  D  S  A  T  Y  Y  C  T  R  D

AGGGGGCTACGGTTCTACTTTGACTACTGGGGCCAAGGCACCACTCTCACAGTCTCCTCA
 R  G  L  R  F  Y  F  D  Y  W  G  Q  G  T  T  L  T  V  S  S
```

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Construction of A5B7 chimeric heavy chain gene.

Fig. 2

Human IgG heavy chain gene

A5B7 murine heavy chain gene
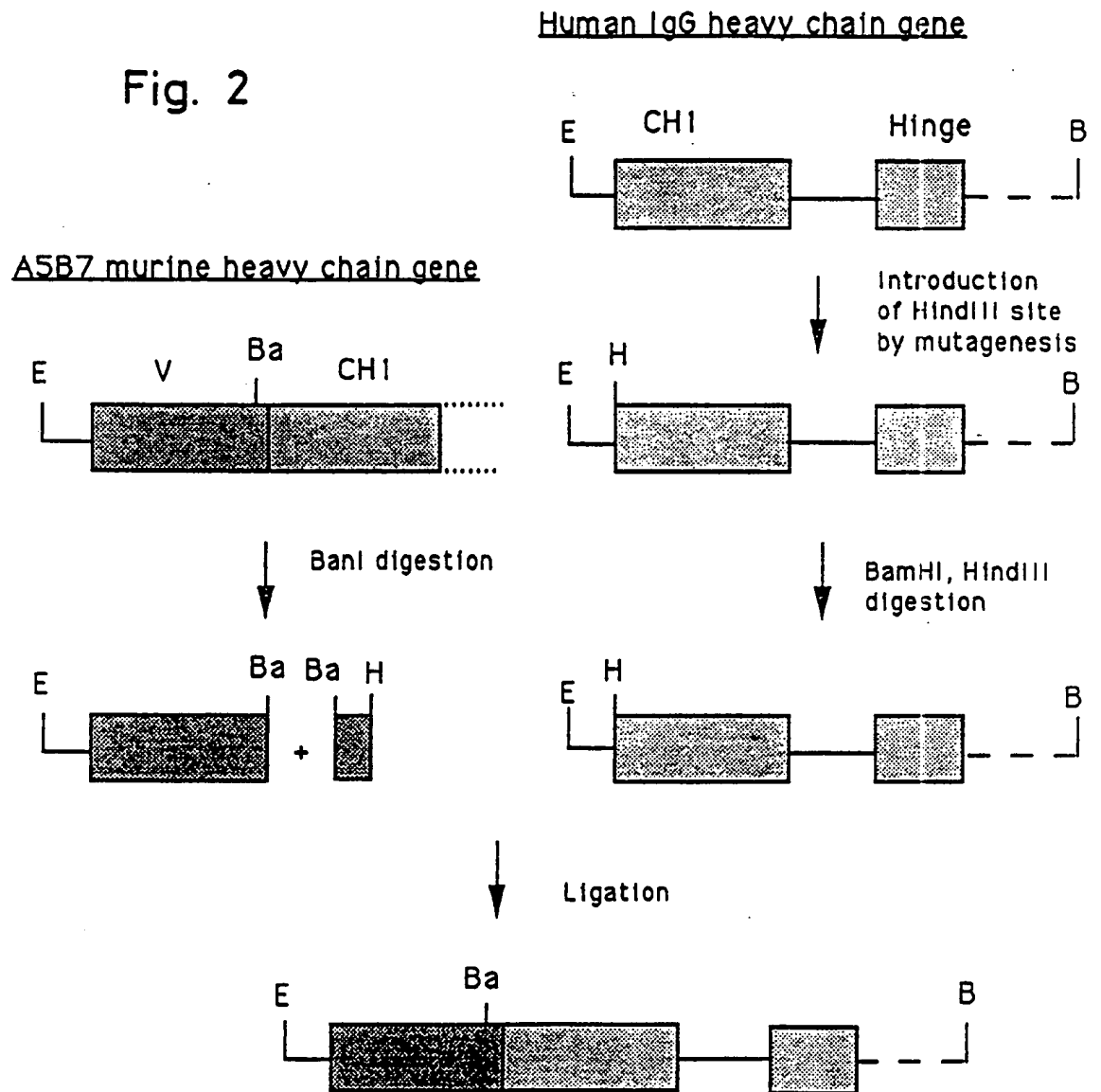
E = EcoRI
B = BamHl
H = HindlII
Ba = Banl

Construction of A5B7 chimeric light chain gene.
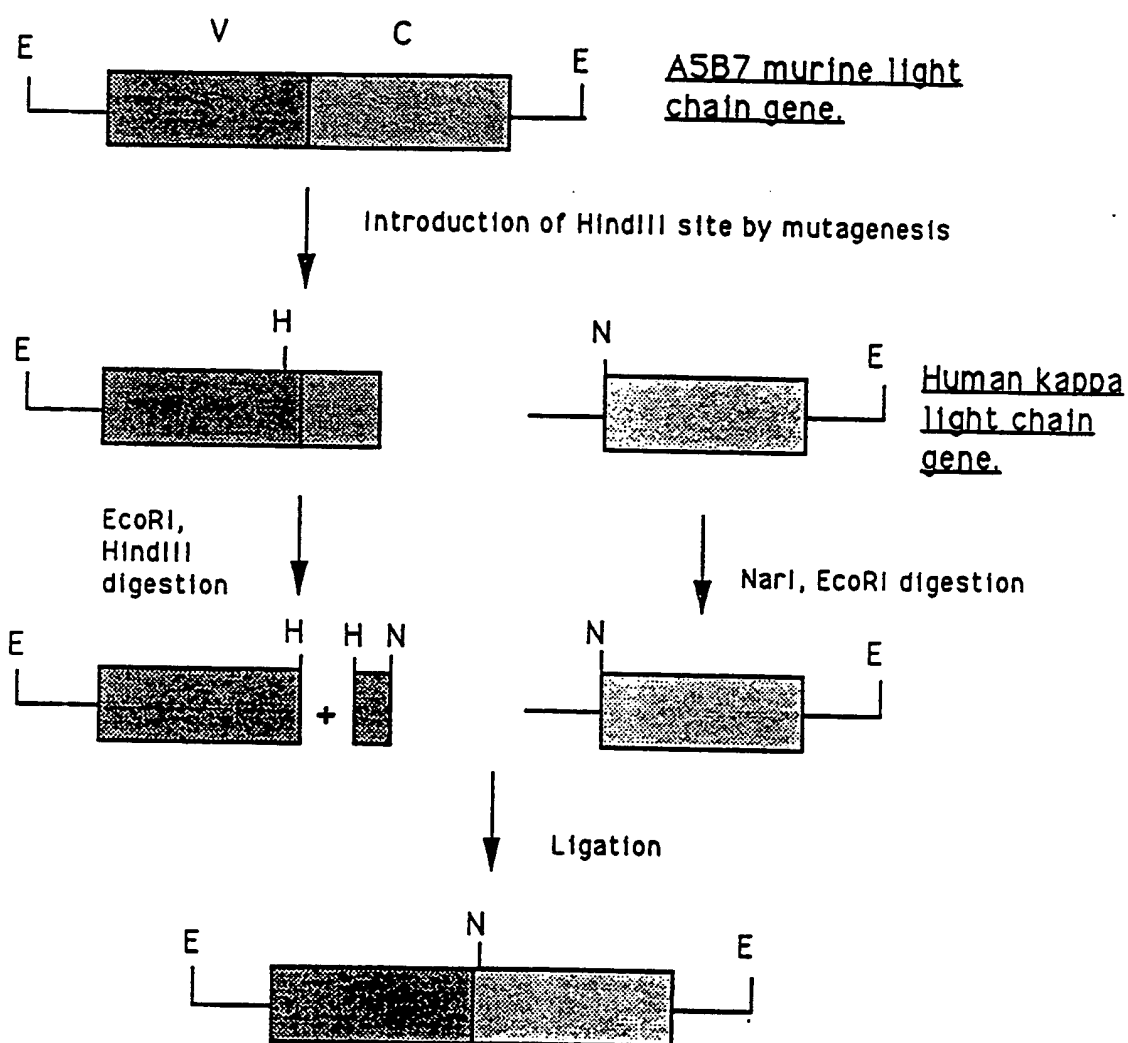
Fig. 3

E = EcoRI
H = HindIII
N = NarI

Fig. 4
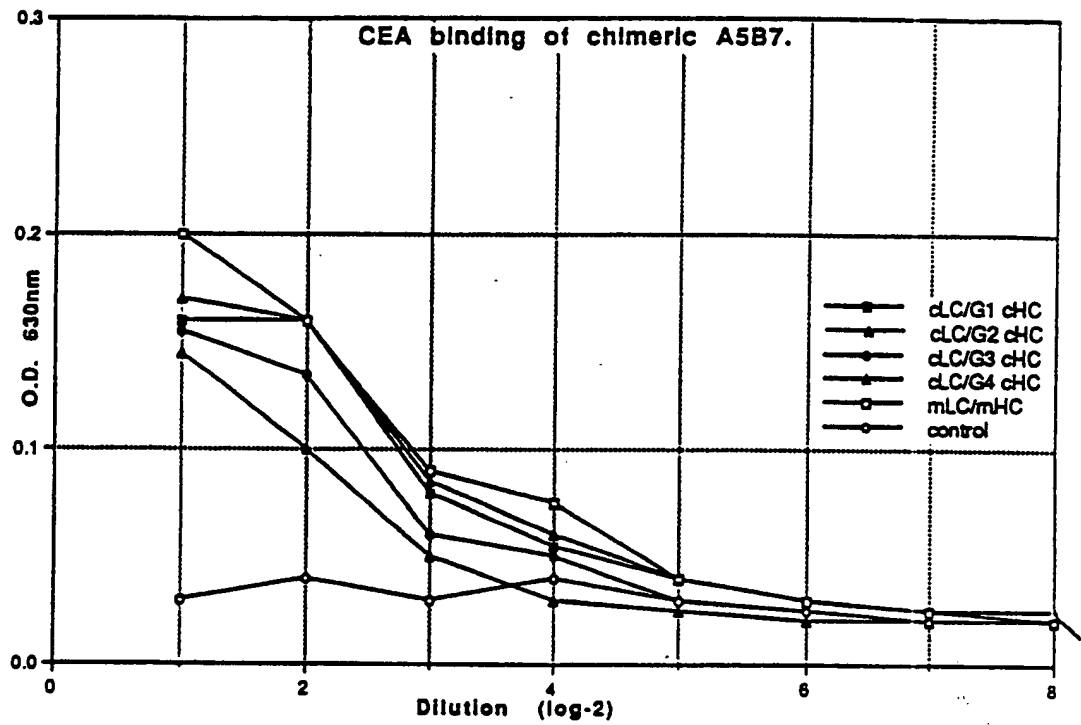
cHC = chimeric heavy chain.

cLC = chimeric light chain.

mHC = mouse heavy chain.

mLC = mouse light chain.

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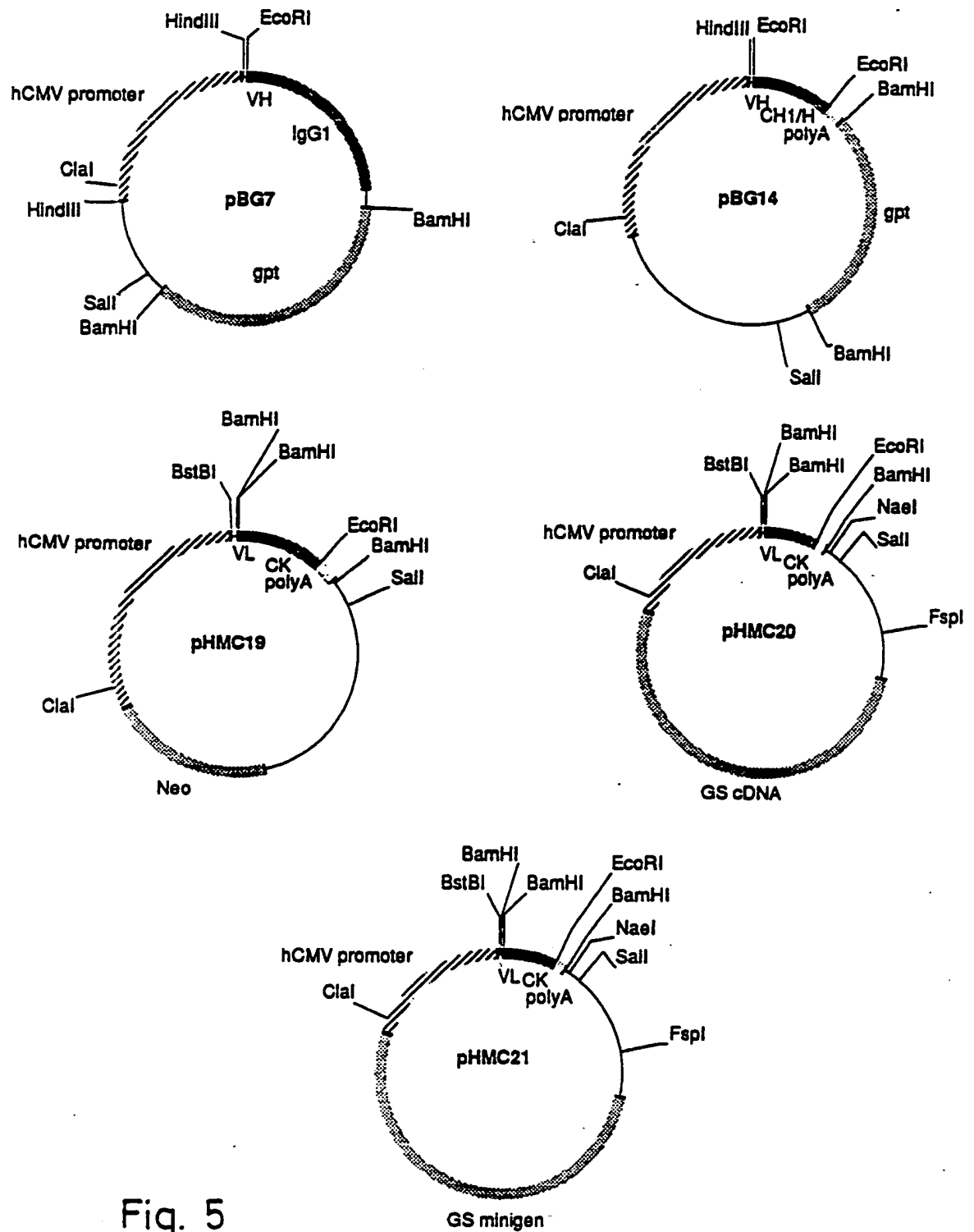
Fig. 5

VECTORS FOR cA5B7 EXPRESSION

Fig. 6
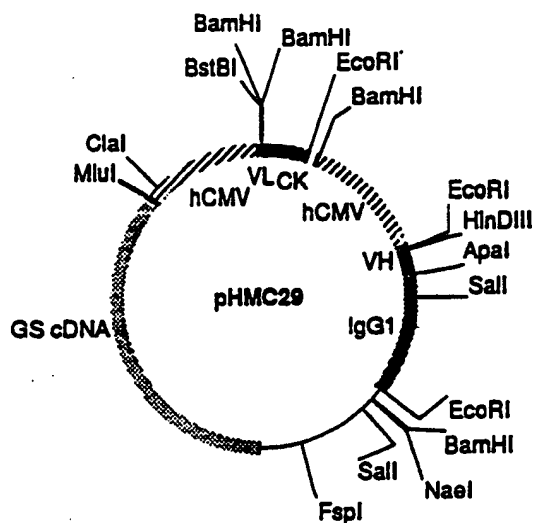
GS vector for cL-cIgG1 expression in NS0 cells
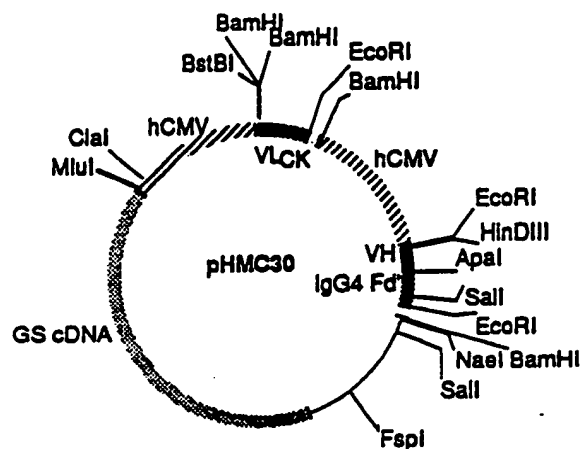
GS vector for cL-cIgG4Fd' expression in NS0 cells
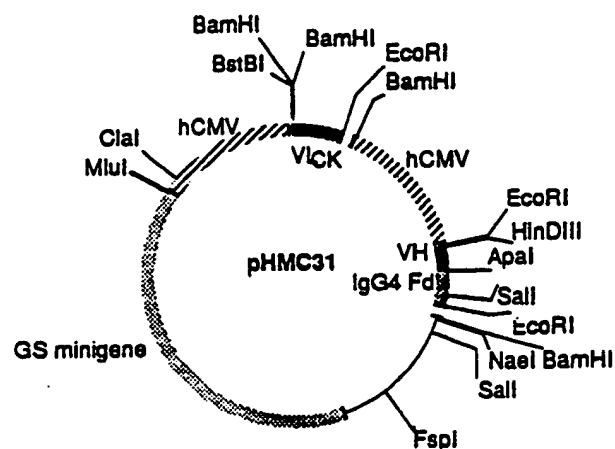
GS vector for cL-cIgG4Fd' expression in CHO cells

GS VECTORS FOR cA5B7 IgG1 AND cA5B7 Fab'

Purification and cross-linking of A5B7 cFab'
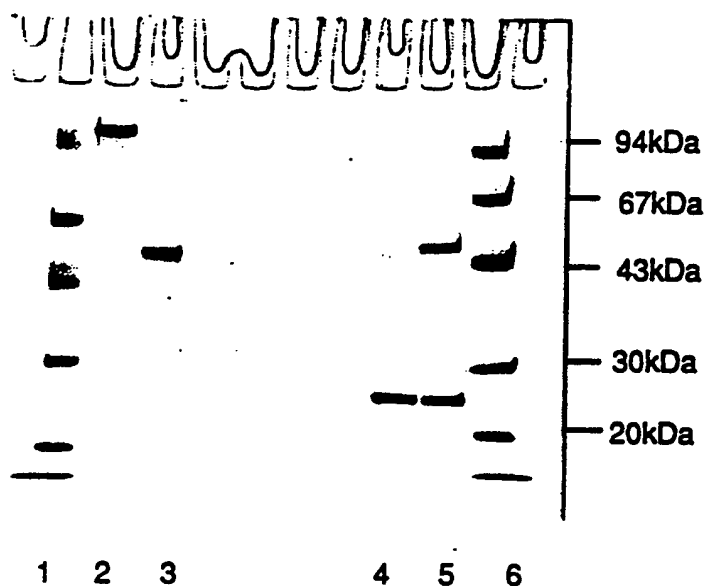
1. Molecular weight markers, non-reduced
2. A5B7 cDFM, non-reduced
3. A5B7 cFab', non-reduced
4. A5B7 cFab', reduced
5. A5B7 cDFM, reduced

10-20% acrylamide gradient gel, Coomassie blue stained

Fig. 7
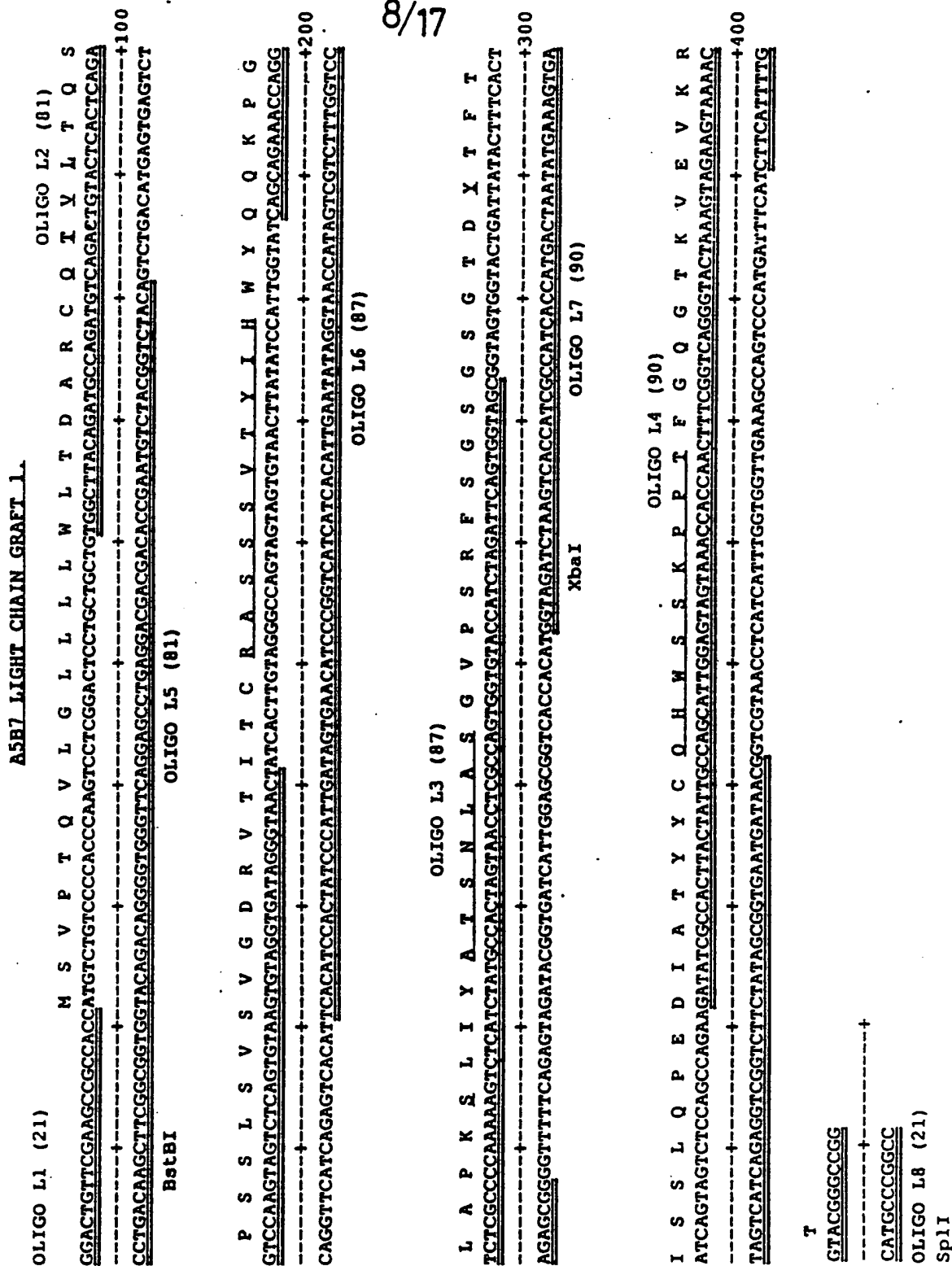
Fig. 8

DNA AND PROTEIN SEQUENCE FOR
gL1-A5B7 VARIABLE REGION

A5B7 LIGHT CHAIN GRAFT 2

OLIGO L1 (21)

M S V P T Q V L G L L L L W L T D A R C Q T Y L T Q S

OLIGO L2 (81)

BstBI

OLIGO L5 (81)

P S S L S V S V G D R V T M T C R A S S S V T Y I H W Y Q Q K P G

OLIGO L3A (87)

OLIGO L6A (87)

L A P K S W I Y A T S N L A S G V P S R F S G S G S G T D Y T L T

OLIGO L7A (90)

XbaI

I S S L Q P E D I A T Y Y C Q H W S S R P T F G Q G T K V E V K R

OLIGO L4 (90)

OLIGO L8 (21)

SplI

Fig. 9

DNA AND PROTEIN SEQUENCE FOR
gL2-A5B7 VARIABLE REGION

A5B7 HEAVY CHAIN GRAFT 1

OLIGO (I -1) H1 (21)

M E W S W V F L F F L S V T T G V H S E V Q L L E S G

OLIGO H5 (90)

Hind III

OLIGO H2 (96)

G G L V Q P G G S L R L S C A T S G F T F T D Y Y M N W V R Q A P

OLIGO H6 (96)

OLIGO H3 (96)

G K G L E W L G F I G N K A N G Y T T E Y S A S V K G R F T I S R

XhoI

OLIGO H7 (96)

D K S K S T L Y L Q M N G L Q A E V S A I Y Y C T R D R G L R E Y F

OLIGO H4 (95)

D Y W G Q G T L V T V S S A S T K G P

OLIGO I -1 H8   ApaI

Fig. 10

DNA AND PROTEIN SEQUENCE FOR
gH1-A5B7 VARIABLE REGION

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A5B7 HEAVY CHAIN GRAFT 2.

OLIGO (L-1) H1 (21)

M E W S W V F L F L S V T T G V H S E V Q L L E S G

GCGGCAAGCTTGCCGCCACCATGGAATGAGCTGGGTTCTTTCTCTCGTCACTAACTACAGAGGTGCATTCTGAGGTGCAGCTGCTGGAGTCTG

CGCGCGTTCGAACGGCGGTGGTACCTTACTCGACCCAGAAAGAGAAGAGCAGTCATTGATGTCCTCAGGTAAGACTCCACGTCGACGACCTCAGAC

HInd III   OLIGO H5 (90)

100

OLIGO H2 (96)

G G L V Q P G G S L R L S C A I S G F T F T D Y Y M N W V R Q A P

GAGGAGGACTGGTGCAGCCTGGAGGATCTCTGAGACTCTGTCGTGTGCAACATCTGGATTCACCTTCACAGACTACTACATGAATTGGGTGAGACAGGCACC

CTCCTCCTGACCACGTCGGACCTCCTAGAGACTCTGAGACAGCACACGTTGTAGACCTAAGTGGAAGTGTCTGATGATGTACTTAACCACTCTGTCCGGTGG

OLIGO H3 (96)   200

G K G L E W L G E I G N K A N G Y T T E Y S A S V K G R F T I S R

TGGAAAGGGACTCGAGTGGCTGGGCTTCATCGGAAATAAGGCAAATGGATACACAACAGAGTACTCTGCATCGTGAAGGGAAGATTCACAATTCCAGA

ACCTTTCCCTGAGCTCACCGACCCGAAGTAGCCTTTATTCCGTTTACCTATGTGTTGTCTCATGAGACGTAGACACTTCCCTTCTAAGTGTTAAGGTCT

XhoI   OLIGO H6 (96)   300

D K S K S T L Y L Q M N T L Q A E D S A I Y Y C T R D R G L R F Y F

GACAAGAGCAAGTCCACACTGTACCTGCAGATGAATACACTGCAGGCAGAGGACTCTGCAATTACTACTACTGTACAAGAGACAGAGGACTGAGATTCTACT

CTGTTCTCGTTCAGGTGTGACATGGACGTCTACTTATGTGACGTCCGTCTCCTGAGACGTTAAATGATGACATGTTCTCGTCTCCTGACTCTAAGATGA

OLIGO H7 (96)   400

D Y W G Q G T L V T V S S A S T K G P

TCGGACTACTGGGGTCAGGGAACACTGGTGACAGTGTCTTCTGCCTCAACGAAGGGCCCGCGGGC

AGCTGATGACCCCCTGTCCCTTGTGACCACTGTCACAGAAGACGGAGTTGCTTCCCGGGCGCCCG

OLIGO L-1 H8 ApaI

Fig. 11

DNA AND PROTEIN SEQUENCE FOR
gH2-A5B7 VARIABLE REGION

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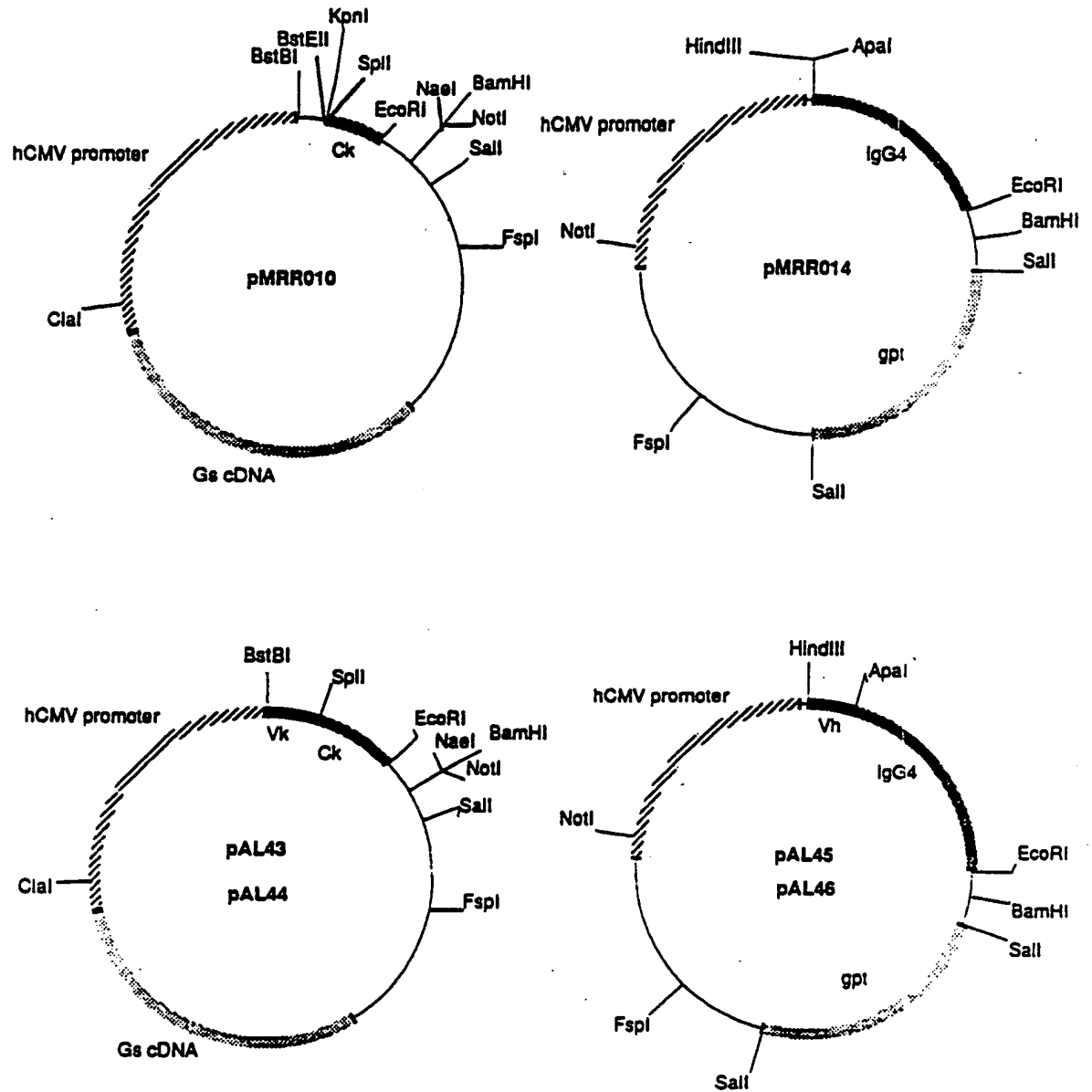
Fig. 12

VECTORS FOR gA5B7
EXPRESSION

## CEA binding A5B7 chimeric/grafted hybrid
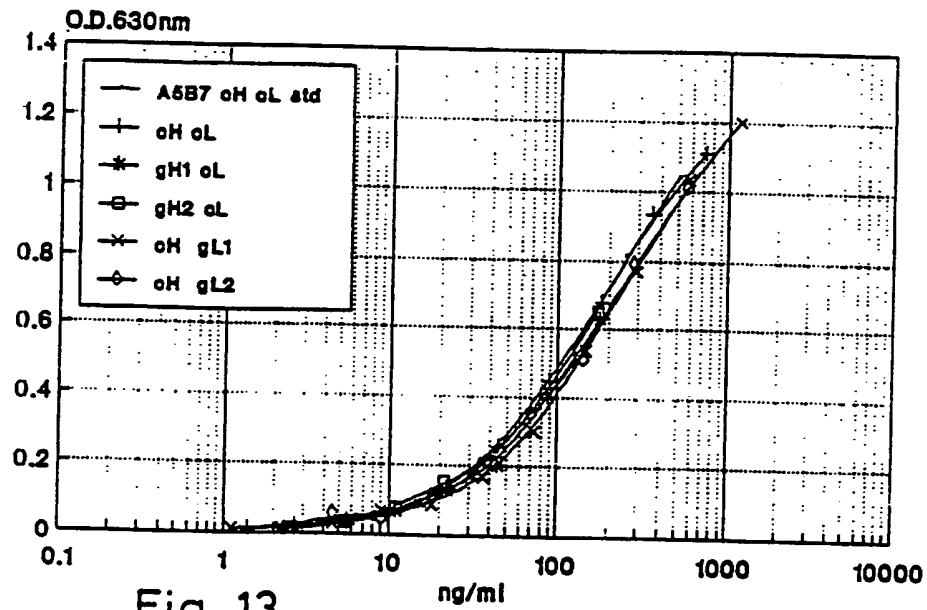
Fig. 13

CEA Binding A5B7 gHgL
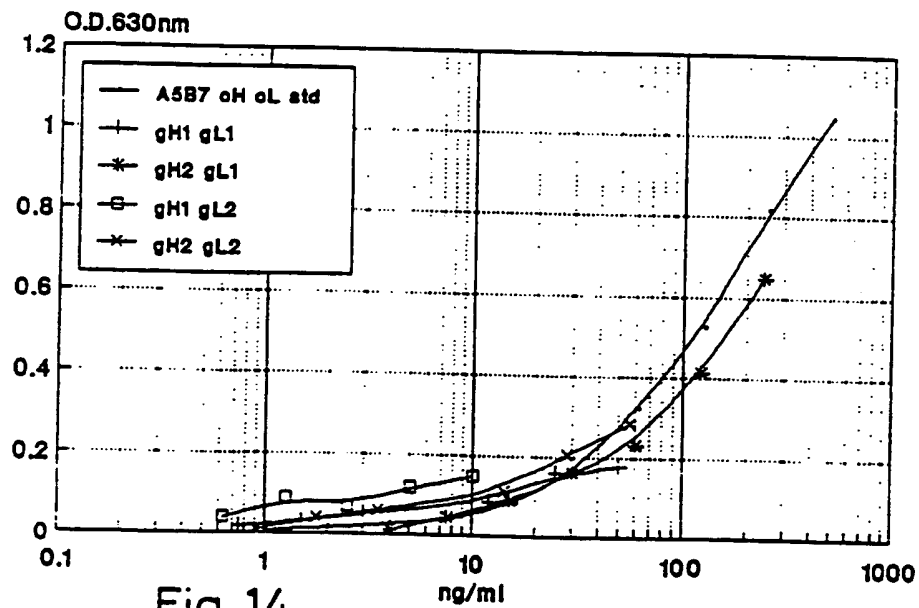
Fig. 14

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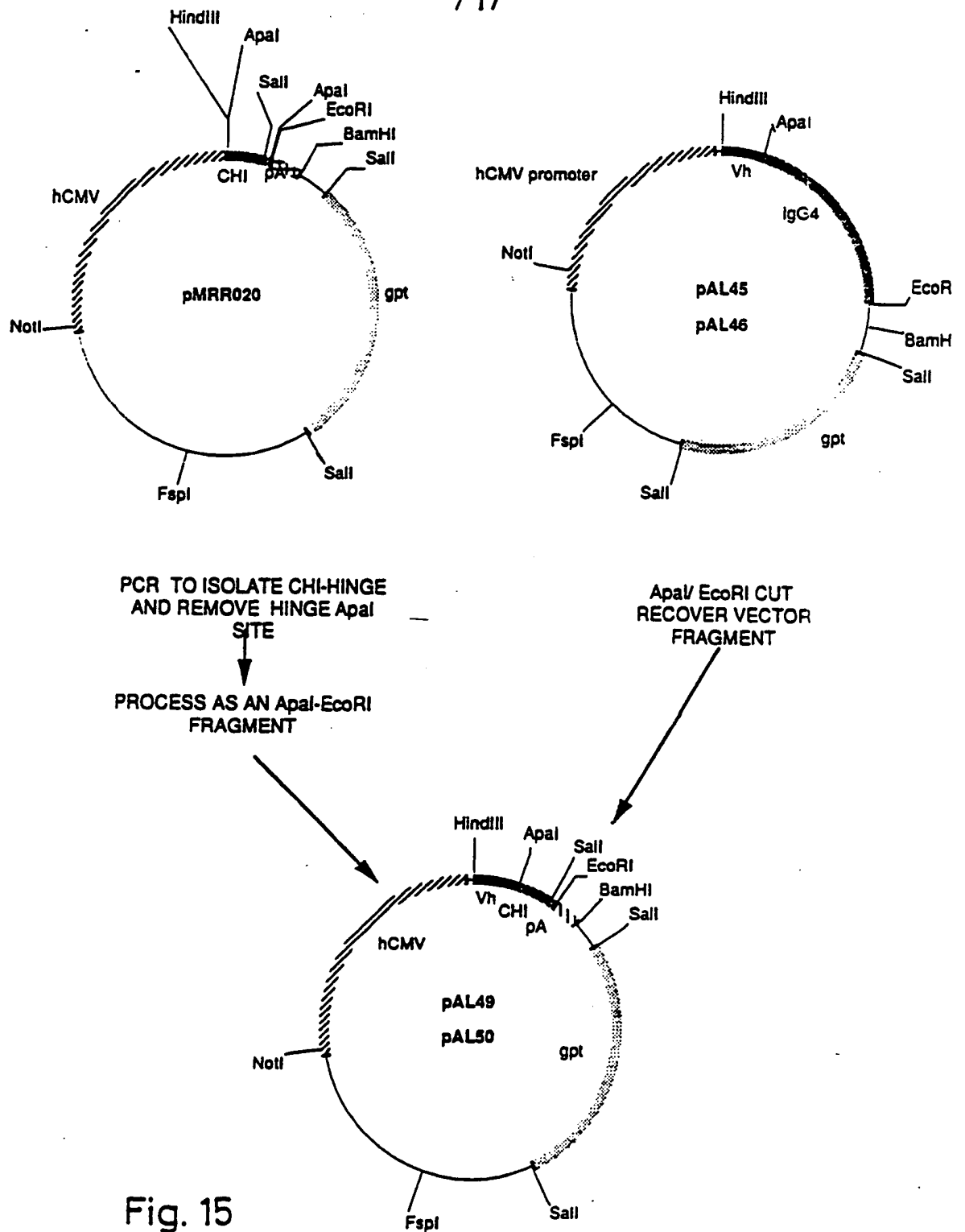
Fig. 15

VECTORS FOR gA5B7 Fab'
EXPRESSION

NOT TO SCALE

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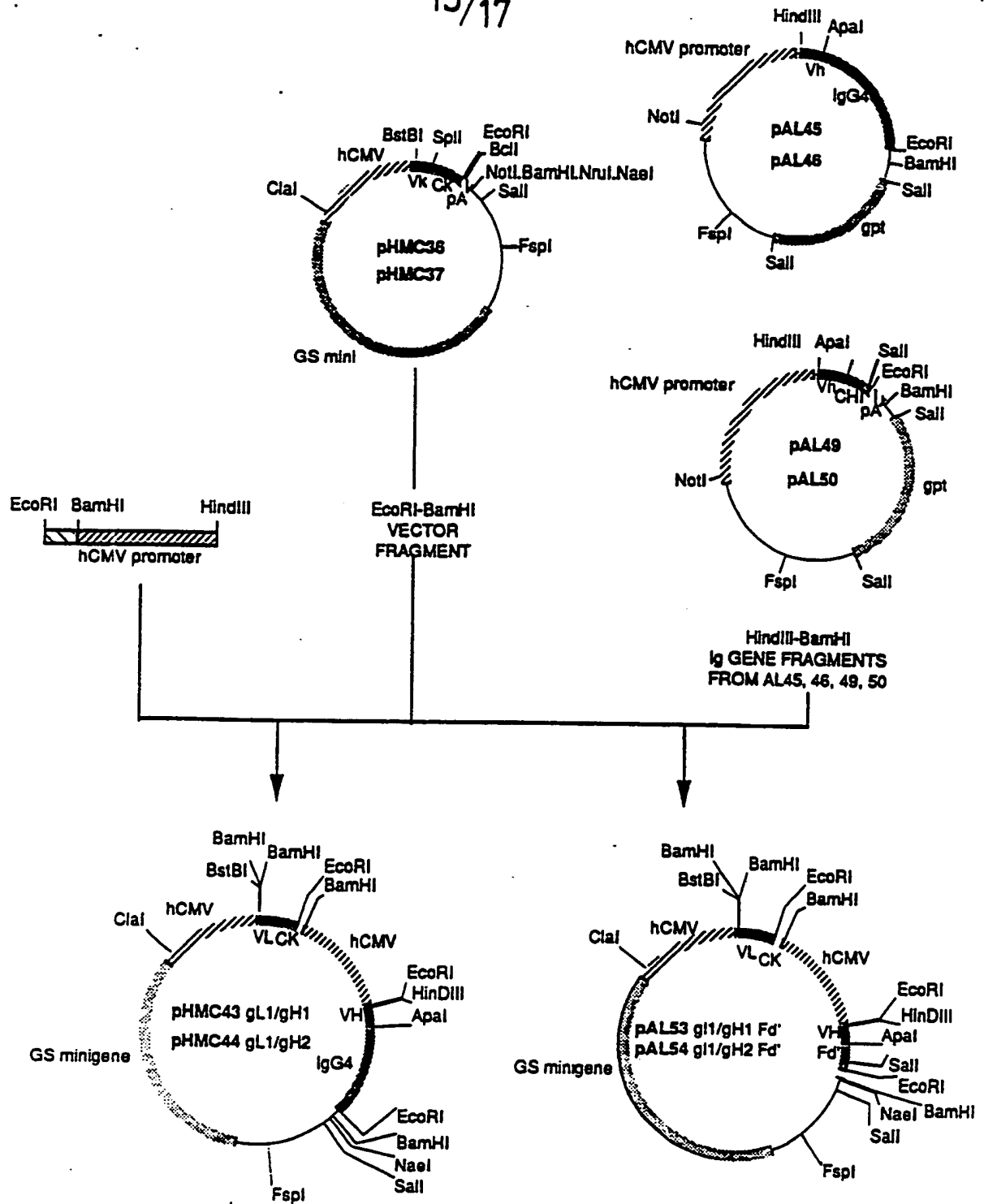
Fig. 16

VECTORS FOR gA5B7 CHO CELL
EXPRESSION

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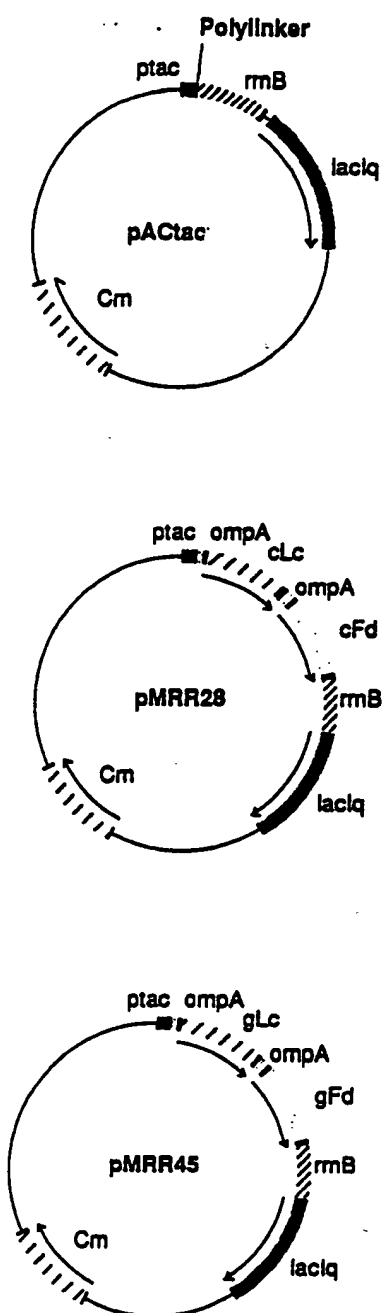
Fig. 17

Maps of A5B7 Fab' E.coli expression plasmids

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CEA binding assay on E.coli supernatants containing A5B7 chimeric Fab'
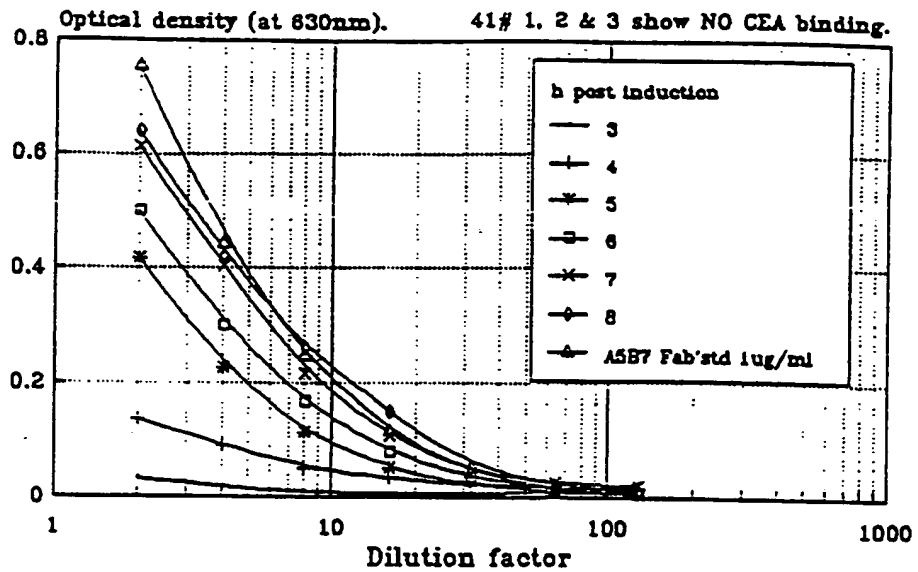

Fig. 18

## CEA binding assay on E.coli supernatants containing A5B7 grafted Fab'
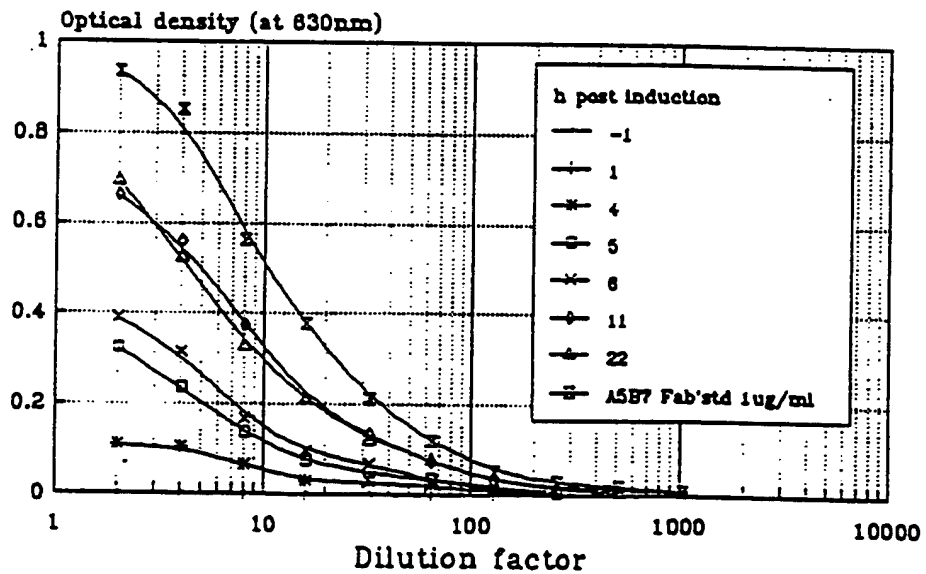
Fig. 19

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 91/01108

I. CLASSIFICATION F SUBJECT MATTER (if several classification symbols apply, indicate all)

According to International Patent Classification (IPC) or to both National Classification a: C

IPC5: C 12 P. 21/08, A 61 K 39/395, C 12 N 15/13, C   K 15/28

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
IPC5	A 61 K; C 12 P; C 12 N

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category[*]	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
Y	WO, A1, 8910140 (CANCER RESEARCH CAMPAIGN TECHNOLOGY LIMITED) 2 November 1989, see page 7, lines 3-13 and 21-24 and examples	1-6,15-21
A	Chemical Abstracts, volume 110, no. 15, 10 April 1989, (Columbus, Ohio, US), Barnett P et al.: "Tumor localization usin monoclonal antibodies. II. Biodistribution of A5B7/indium-111 chelates", see page 363, abstract 131464s, & Int. J. Cancer (Suppl. 3) 1988, (), 34- 37	1-21
A	Br. J. Cancer, vol. 54, 1986, P.J. Harwood et. al.: "Mapping epitope characteristics on carcinoembryonic antigen ", see page 75 - page 82	1-21

* Special categories of cited documents:[10]

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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"Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
11th October 1991	25 10 91

International Searching Authority	Signature of Authorized Officer
EUROPEAN PATENT OFFICE	

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT	(CONTINUED FROM THE SECOND SHEET)	
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	WO, A2, 8901783 (CELLTECH LIMITED) 9 March 1989, see the whole document --	1-6,15-21
Y	Nature, vol. 332, March 1988, L Riechmann et al.: "Reshaping human antibodies for therapy ", see page 323 - page 327 --	1-6,15-21
Y	EP, A1, 0323806 (CIBA-GEIGY AG) 12 July 1989, see page 5 --	1-6,15-21
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A	Proc. Natl. Acad. Sci., vol. 86, December 1989, C Queen et al.: "A humanized antibody that binds to the interleukin 2 receptor ", see page 10029-10033 --	1-21
A	Science, vol. 239, 1988, M Verhoeyen et al.: "Reshaping Human Antibodies: Grafting an Antilysozyme Activity ", see page 1534 - page 1536 --	1-21

ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.PCT/GB 91/01108

SA   49262

This annex lists the patent family members relating to the patent documents cited in the above-mentioned International search report.
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		AU-D-	2301688	31/03/89
		AU-D-	2421888	31/03/89
		EP-A-	0329755	30/08/89
		EP-A-	0347433	27/12/89
		EP-A-	0348442	03/01/90
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		JP-T-	2501191	26/04/90
		JP-T-	2501800	21/06/90
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		WO-A-	89/01974	09/03/89
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		JP-A-	2154696	14/06/90

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